U.S. DEPARTMENT OF COMMERCE
National Technical Information Service

PB80-112964

The Disinfection of Drinking Water

(U.S.) National Research Council, Washington, DC

Prepared for

Environmental Protection Agency, Washington, DC Office of Drinking Water

Mar 79

Criteria and Standards Divisi
Office of Drinking Water

PB80-112964

THE DISINFECTION OF DRINKING WATER

prepared for the
Environmental Protection Agency

by the
Subcommittee on Efficacy of Disinfection
of the
Safe Drinking Water Committee

Board on Toxicology and Environmental Health Hazards
Assembly of Life Sciences, National Research Council

NATIONAL ACADEMY OF SCIENCES
Washington, D.C. 1979

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U. S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

TECHNICAL REPORT DATA

(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO. PB80-112964
4. TITLE AND SUBTITLE The Disinfection of Drinking Water		5. REPORT DATE March, 1979
		6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S)		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS National Academy of Sciences 2101 Constitution Ave. Washington, D.C.		10. PROGRAM ELEMENT NO.
		11. CONTRACT/GRANT NO. 68-01-3169
12. SPONSORING AGENCY NAME AND ADDRESS Office of Drinking Water Environmental Protection Agency Washington, D.C. 20460		13. TYPE OF REPORT AND PERIOD COVERED Final
		14. SPONSORING AGENCY CODE

15. SUPPLEMENTARY NOTES

16. ABSTRACT

The current status of theoretically possible methods for disinfecting drinking water ~~is~~ reviewed. The specific biocidal activity of each of the disinfectants is considered, as well as information (or lack of it) on the practical application and reliability of the methods.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT Release unlimited	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES 234
	20. SECURITY CLASS *(This page)*	22. PRICE A11-A01

EPA Form 2220-1 (9-73)

NOTICE: The project that is the subject of this report was approved by the Governing Board of the National Research Council, whose members are drawn from the Councils of the National Academy of Sciences, the National Academy of Engineering and the Institute of Medicine. The Members of the Committee responsible for the report were chosen for their special competences and with regard for appropriate balance.

This report has been reviewed by a group other than the authors according to procedures approved by a Report Review Committee consisting of members of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine.

At the request of and funded by the U.S. Environmental Protection Agency Contract No. 68-01-3169

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THE DISINFECTION OF DRINKING WATER

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THE DISINFECTION OF DRINKING WATER

The goal of disinfection of public water supplies is the elimination of the pathogens that are responsible for waterborne diseases. The transmission of diseases such as typhoid and paratyphoid fevers, cholera, salmonellosis, and shigellosis can be controlled with treatments that substantially reduce the total number of viable microorganisms in the water.

While the concentration of organisms in drinking water after effective disinfection may be exceedingly small, sterilization (i.e., killing all the microbes present) is not attempted. Sterilization is not only impractical, it cannot be maintained in the distribution system. Assessment of the reduction in microbes that is sufficient to protect against the transmission of pathogens in water is discussed below.

Chlorination is the most widely used method for disinfecting water supplies in the United States. The near universal adoption of this method can be attributed to its convenience and to its highly satisfactory performance as a disinfectant, which has been established by decades of use. It has been so successful that freedom from epidemics of waterborne diseases is now virtually taken for granted. As stated in Drinking Water and Health (NAS, 1977), "chlorination is the standard of disinfection against which others are compared."

However, the discovery that chlorination can result in the formation of trihalomethanes (THM's) and other halogenated hydrocarbons has prompted the reexamination of available disinfection methodology to determine alternative agents or procedures (Morris, 1975).

The method of choice for disinfecting water for human consumption depends on a variety of factors (Symons *et al*., 1977). These include:

- its efficacy against waterborne pathogens (bacteria, viruses, protozoa, and helminths);
- the accuracy with which the process can be monitored and controlled;
- its ability to produce a residual that provides an added measure of protection against possible posttreatment contamination resulting from faults in the distribution system;
- the aesthetic quality of the treated water; and
- the availability of the technology for the adoption of the method on the scale that is required for public water supplies.

Economic factors will also play a part in the final decision; however, this study is confined to a discussion of the five factors listed above as they apply to various disinfectants.

The propensity of various disinfection methods to produce by-products having effects on health (other than those relating to the control of infectious diseases) and the possibility of eliminating or avoiding these undesirable by-products are also important factors to be weighed when making the final decisions about overall suitability of methods to disinfect drinking water. The subcommittee has not attempted to deal with these problems since the chemistry of disinfectants in water and the toxicology of expected by-products have been studied by other subcommittees of the Safe Drinking Water Committee.

REPORT ORGANISATION

The general considerations noted in the immediately following material should be borne in mind when considering each method of disinfection. Available information on the obvious major candidates for drinking water disinfection -- chlorine, ozone, chlorine dioxide, iodine and bromine -- is then evaluated for each method individually in the following sections. Other less obvious possibilities are also examined to see if they have been overlooked unjustly in previous studies or if it might be profitable to conduct further experimentation on them. Disinfection by chloramines is dealt with in parallel with that effected by chlorine because of the close relationship the former has to chlorine disinfection under conditions that might be normally encountered in drinking water treatment.

The evlauations in this report are not exhaustive literature reviews but, rather, are selections of the studies which, in the judgement of the committee provide the most accurate and relevant information on the biocidal activities of each method of disinfection. The analytical methods that are described in this report are those that are most likely to be used by persons involved in disinfection research or water treatment. A review of all existing analytical methods, some of which may be more sophisticated than those described below, would be impractical within the constraints of time and space available and is not within the scope of this document.

After the methods of disinfection are examined individually their major characteristics and biocidal efficacy are compared by means of summary tables and c.t (concentration, mg/l, times contact time, minutes) values required for similar inactivations under identical conditions. The conclusions of the study are then recorded on the basis of this evidence.

GENERAL ASPECTS OF DISINFECTION

In any comparison of disinfection methods certain considerations should be discussed at the outset since they are relevant to most, if not all, methods. The quality of the raw water (i.e., its content of solids and material that will react with the disinfectant), treatment of the water prior to disinfection, and the manner in which the disinfectant is applied to the water will directly affect the efficacy of all disinfectants. Equally applicable to all methods are appropriate standards for verifying the adequacy of disinfection, differences in response to disinfectants between organisms that were obtained directly from the field and those that have been acclimated to laboratory culture, and the maintenance of potability from treatment plant to the consumer's tap. The use of chlorination as presented in examples in the following pages does not imply that it is necessarily the method of choice. Rather, this method has been studied more thoroughly than other methods.

Raw Water Quality

In addition to potential pathogens, raw water may contain contaminants that may interfere with the disinfection process or may be undesirable in the finished product. These contaminants include inorganic and organic molecules, particulates, and other organisms, e.g., invertebrates. Variations among these contaminants arise from differences in regional geochemistry and between ground- and surface-water sources.

Disinfectant Demand

Many inorganic and organic molecules that occur in raw water exert a "demand", i.e., a capacity to react with and consume the disinfectant. Therefore, higher "demand" waters require a greater dose to achieve a specific concentration of the active species of disinfectant. This demand must be satisfied to ensure adequate biocidal treatment.

Ferrous ions, nitrites, hydrogen sulfide, and various organic molecules exert a demand for oxidizing disinfectants such as chlorine. The bulk of the nonparticulate organic material in raw water occurs as naturally derived humic substances, i.e., humic, fulvic, and hymatomelanic acids, which contribute to color in water. The structure of these molecules is not yet fully understood. However, they are known to be polymeric and to contain aromatic rings and carboxyl, phenolic, alcoholic hydroxyl, and methoxyl functional groups. Humic substances, when reacting with and consuming applied chlorine, produce chloroform ($CHCl_3$) and other THM's. Water, particularly surface waters, may also contain synthetic organic molecules whose demand for disinfectant will be determined by their structure. Ammonia and amines in raw water will react with chlorine to yield chloramines that do have some biocidal activity, unlike most products of these side reactions. If chlorination progresses to the breakpoint, i.e., to a free-chlorine residual, these chloramines will be oxidized causing more added chlorine to be consumed before a specific free-chlorine level is achieved. This phenomenon is discussed more fully below.

The nature of the demand reactions varies with the composition of the water and the disinfectant. Removal of the demand substances leaves a water with a lower requirement for a disinfectant to achieve an equivalent degree of protection against transmission of a waterborne disease.

Physical and Chemical Treatments

Various treatments applied to raw water to remedy undesirable characteristics, e.g., color, taste, odor, or turbidity, may affect the ultimate microbiological quality of the finished water. Microorganisms may be physically removed or the disinfectant demand of the water altered.

Presedimentation to remove suspended matter, coagulation with alum or other agents, and filtration reduce the organic material in the raw water and, thus, the disinfectant demand. Removal of ferrous iron similarly reduces the demand for oxidizing disinfectants as will aeration, which eliminates hydrogen sulfide. Prechlorination to a free chlorine residual is practiced early in the treatment sequence as one method to alter taste- and odor-producing compounds, to suppress growth of organisms in the treatment plant, to remove iron and manganese, and to reduce the interference of organic compounds in the coagulation process.

The necessity for these treatments or others is determined by the characteristics of the raw water. The selection of one of the various methods to achieve a particular result will be based upon cost-effectiveness in the particular situation. When chlorination is used, the application or point of application in the treatment sequence of some of the above-mentioned procedures can affect the undesirable THM content of the finished water.

Reduction of precursors in raw water by coagulation and settling prior to chlorination reduces final THM production (Hoehn et al., 1977; Stevens et al., 1975). The Louisville Water Company reduced THM concentrations leaving the plant by 40%-50% by shifting the point of chlorination from the presedimentation basin to the coagulation basin (Hubbs et al., 1977). The available information on these variations is limited, and a universally applicable procedure cannot be recommended in view of the diverse treatments required for different raw waters.

Particulates and Aggregates

To inactivate organisms in water, the active chemical species must be able to reach the reactive site within the organism or on its surface. Inactivation will not result if this cannot occur. Microorganisms may acquire physical protection in water as a result of their being adsorbed to the enormous surfaces provided by clays, silt, and organic matter or to the surfaces of solids created during water treatment, e.g., aluminum or ferric hydrated oxides, calcium carbonate, and magnesium hydroxide. Viruses, bacteria, and protozoan cysts may be adsorbed to these surfaces. Such particles, with the adsorbed microorganisms, may aggregate to form clumps, affording additional protection. Organisms themselves may also aggregate or clump together so that organisms that are on the interior of the clump are shielded from the disinfectant and are not inactivated. Organisms may also be physically embedded within particles of fecal material, within larger organisms such as nematodes, or, in the case of viruses, within human body cells that have been discharged in fecal material.

To disinfect water adequately it must have been pretreated, when necessary, to reduce the concentration of solid materials to an acceptably low level. The primary drinking water turbidity standard of 1 Nephelometric Turbidity Unit (NTU) is an attempt to assure that the concentration of particulates is compatible with current disinfection techniques. Where it is possible to obtain lower turbidities this is desirable.

Disinfection studies in which the complications of adsorbed organisms, aggregation, or embedment were thought to occur were excluded from this study. The conclusions in this report should not be extrapolated to such situations as the disinfection of turbid or colored waters.

The Importance of Residuals

Water supplies are disinfected through the addition or dosage of a chemical or physical agent. With a chemical agent, such as a halogen, a given dosage should theoretically impart a predetermined concentration (residual) of the active agent in the water. From a practical point of view, most natural waters exert a "demand" for the disinfectant, as discussed above, so that the residual in the water is less than the calculated amount based on the dosage. The decrease in residual, which is caused by the demand, is rapid in most cases, but it may be prolonged until the residual eventually disappears. In addition, the chemical agent may decompose spontaneously, thereby yielding substances having little or no disinfection ability and exerting no measurable residual. For example, ozone not only reacts with substances in water that exert a demand, but it also decomposes rapidly. To achieve microbial inactivation with a chemical agent, a residual must be present for a specific time. Thus, the nature and level of the residual, together with time of exposure, are important in achieving disinfection or microbial inactivation. Because the nature of the dosage-residual relationship for natural waters has not been and possibly cannot be reliably defined, the efficacy of disinfection with a chemical agent must be based on a residual concentration/time-of-exposure relationship.

Residual measurements are important and useful in controlling the disinfection process. By knowing the residual-time relationship that is required to inactivate pathogenic or infectious agents, one can adjust the dosage of the disinfecting agent to achieve the residual that is required for effective disinfection with a given contact time. Thus, the effectiveness of the disinfection process can be controlled and/or judged by monitoring or measuring the residual.

Following disinfection of a water supply at a treatment plant, the water is distributed to the consumers. A persistent residual is important for continued protection of the water supply against subsequent contamination in the distribution system. Accidental or mechanical failures in the distribution system may result in the introduction of infectious agents into the water supply. In the presence of a residual, disinfection will continue and, as a result, offer continued protection to the users. Physical agents such as radiation may provide effective disinfection during application, but they do not impart any persistent residual to the water.

The dosage of a chemical agent that is used to effect microbial inactivation should not be so great that it imparts a health hazard to the water consumer. From another point of view, the aesthetic quality of the finished water should not be impaired by the dosage of the chemical agent or the residual that is required for effective disinfection. These qualities might include discoloration of water from potassium permanganate ($KMnO_4$) or iodine or problems of taste and odor from excessive chlorine.

Application of the Disinfectant

Optimum inactivation occurs when the disinfectant is distributed uniformly throughout the water. To disperse the chemical disinfectant when it is added to the water, it must be mixed effectively to assure that all of the water, however small the volume, receives its proportionate share of the chemical. Additions of a disinfectant at points in a flowing water stream, e.g., from submerged pipes, is seldom adequate to assure uniform concentration. In such cases, mechanical mixing devices are needed to disperse the disinfectant throughout the water. Disinfection by radiation treatment also requires good mixing to bring all of the water within the effective radiation distance.

Microbiological Considerations[1]

Comparison of the biocidal efficacy of disinfectants is complicated by the need to control many variables, a need not realized in some early studies. Halogens in particular are significantly affected by the composition of the test menstruum and its pH, temperature, and halogen demand. For very low concentrations of halogen to be present over a testing period, halogen demand must be carefully eliminated. Different disinfectants may have different biocidal potential. In earlier work, analytical difficulties may have precluded defining exactly the species present but new techniques allow the species to be defined for most disinfectants. Information on the species of disinfectant actually in the test system should be included in future reports on disinfection studies.

Investigators studying efficacy have usually adopted one of two extremes. some have conducted carefully designed laboratory experiments with controls for as many variables as possible. Certain of these investigators have reduced the temperature to slow the inactivation reactions. Although these experiments yield good basic information and can be used to determine which variables are important, they often have little quantitative relationship to field situations. The other extreme, a field study or reconstruction of field conditions, is difficult to control. Moreover, their results are often not repeatable.

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[1] Nomenclature in this report follows that recommended in the 8th Edition of Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974). Thus, the name of an organism mentioned in the text may not be that used by the author of the work cited.

In addition to the variables noted above, prereaction of chemicals in the test system, the culture history of the organism being used, and the 'clean-up' procedures applied to it may also affect the observed results. Despite these problems there have been some attempts to standardize efficacy testing.

Model Systems and Indicator Organisms

A major factor that influences the evaluation of the efficacy of a particular disinfectant is the test microorganism. There is a wide variation in susceptibility, not only among bacteria, viruses, and protozoa (cyst stage) but also among genera, species, and strains of the microorganism. It is impractical to obtain information on the inactivation by each disinfectant for each species and strain of pathogenic microorganism of importance in water. In addition, interpretation of the data would be confounded by the condition and source of the test microorganism (e.g., the degree of aggregation and whether the organisms were 'naturally occurring' or laboratory preparations), the presence of solids and particulates, and the presence of materials that react with and consume the disinfectant.

The overwhelming majority of the literature on water disinfection concerns the inactivation of model microorganisms rather than the pathogens. These disinfectant model microorganisms have generally been nonpathogenic microorganisms that are as similar as possible to the pathogen and behave in a similar manner when exposed to the disinfectant. The disinfectant model systems are simpler, less fastidious, technically more workable systems that provide a way to obtain basic information concerning fundamental parameters and reactions. The information gained with the model systems can then be used to design key experiments in the more difficult systems. The disinfection model microorganism should be clearly distinguished from the indicator organism. The indicator microorganism, as defined in Drinking Water and Health (NAS, 1977), is a "microorganism whose presence is evidence that pollution (associated with fecal contamination from man or other warm-blooded animals) has occurred."

Following are criteria for the indicator microorganism (Fair and Geyer, 1954):

1. The indicator should always be present when fecal material is present and absent in clean, uncontaminated water.
2. The indicator should die away in the natural aquatic environment and respond to treatment processes in a manner that is similar to that of the pathogens of interest.
3. The indicator should be more numerous than the pathogens.

4. The indicator should be easy to isolate, identify, and enumerate.

Only a restrictive application of the second criterion is necessary for a disinfection model. The response of the test microorganism to the disinfectant must be similar to that of the pathogen that it is intended to simulate. The disinfection model is not meant to function as an indicator microorganism.

During the latter part of the 19th century, investigators, recognized the presence of a group of bacteria which occured in large numbers in feces and wastewater. The most significant member of this group (currently called the coliform group) is *Escherichia coli*. Since the late 19th century, this coliform group has served as an indicator of the degree of fecal contamination of water, and *E. coli* has been used routinely as a disinfection model for enteric pathogens. Butterfield and coworkers (Butterfield *et al*., 1943; Butterfield and Wattie, 1946; Wattie and Butterfield, 1944)) provided information on the inactivation of *E. coli* and other enteric bacterial pathogens with chlorine and chloramines. At pH values above 8.5 all strains of *E. coli* were more resistant to free chlorine than were *Salmonella typhi* strains. At pH values of 6.5 and 7.0, strains of *S. typhi* were more resistant. Only slight differences between the two genera were found when chloramines were used as the disinfectant. The bactericidal activity of chloramine was noticably less than that of free chlorine.

Bacteria of the coliform group, especially _E. coli_, have proved useful as an indicator and disinfection model for enteric bacterial pathogens, but are poor indicators and disinfection models for nonbacterial pathogens. _E. coli_ has been observed to be markedly more susceptible to chlorine than certain enteric viruses and cysts of pathogenic protozoa (Dahling _et al_., 1972; Krusé, 1969).

The bacterial viruses of _E. coli_ have received increased attention as possible disinfection models and indicators of enteric viruses in water and wastewater. At present, the data to justify the bacterial viruses as indicators for enteric viruses are limited and inconsistent. However, there is a growing body of knowledge on the utilization of bacterial viruses as disinfection models.

Hsu (1964) and Hsu _et al_. (1966) first reported the use of the f2 virus as a model for disinfection studies with iodine. They showed that inactivation of both the f2 virus and poliovirus 1 were inhibited by increasing concentrations of iodide ion and that both f2 RNA and poliovirus 1 RNA were resistant to iodination.

Dahling _et al_. (1972) compared the inactivation of two enteric viruses (poliovirus 1 and coxsackievirus A9), two DNA phages (T2 and T5), two RNA phages (f2 and MS2), and _E. coli_ ATCC 11229 under demand-free conditions with free chlorine at pH 6.0. They found enteric viruses to be most resistant to free chlorine followed by RNA phages, E. coli, and the T phages.

Shah and McCamish (1972) compared the resistance of poliovirus 1 and the coliphages f2 and T2 to 4 mg/l combined residual chlorine. The f2 virus was shown to be more resistant to this form of chlorine than poliovirus 1 and T2 coliphage.

Cramer et al. (1976) compared the inactivation of poliovirus 3 (Leon) and f2 with chlorine and iodine in buffered wastewater. Both viruses were treated together in the same reaction flask thereby eliminating any inherent differences due to virus preparations and replicate systems. In wastewater effluent at pH 6.0 and 10.0 with a 30 mg/l dosage of halogen under prereacted (halogen added to wastewater, allowed to react, viruses added at zero time) and dynamic (viruses added to wastewater, halogen added at zero time) conditions, f2 was, in each case, at least as or more resistant to chlorine and iodine than poliovirus 1. The f2 virus appears to be more sensitive to free chlorine but more resistant to combined chlorine than poliovirus 1.

Neefe et al. (1945) observed that the agent of infectious hepatitis was inactivated by breakpoint chlorination (free chlorine) but not completely inactivated by combined chlorine.

Engelbrecht et al. (1975) reported the use of a yeast (Candida parapsilosis) and two acid-fast bacteria (Mycobacterium fortuition and Mycobacterium phlei) may be suitable disinfection models. They observed that the yeast was more resistant to free chlorine than were poliovirus 1 and the enteric bacteria under all conditions tested. The acid-fast bacilli were most resistant.

There is no generally accepted disinfection model for protozoan cysts. In disinfection studies for protozoan diseases, investigators have used the pathogen or its cysts. Work with such systems is, however, generally difficult.

The use of disinfection models provides useful information that is helpful to the comparison of the relative efficiencies of various disinfectants in the laboratory and in controlled field investigations. Strains of *E. coli* have been used extensively as models for enteric pathogenic bacteria. While not as widely accepted, the bacterial viruses of *E. coli* are used as disinfection models for enteric viruses. The difficulty of available methods has limited the number of disinfection studies with protozoan cysts.

Laboratory Cultures versus 'Naturally Occurring' Organisms

The resistance or sensitivity to disinfectants of some bacteria (e.g., *E. coli*) in the laboratory may bear very little resemblance to their responses in nature. This is true in spite of the fact that standardized procedures govern the conditions under which cells are grown, harvested, washed, etc., when they are used as inocula.

Examples of such differences range from Gram-negative bacteria and their comparative resistance to disinfectants in general (Carson et al., 1972; Favero et al., 1971, 1975) to Gram-positive bacterial spores and heat resistance (Bond et al., 1973) and to halogen resistance of *Entamoeba histolytica* cysts from simian hosts as opposed to those grown in *in-vitro* systems (Stringer et al., 1975). Presumably, the mechanisms creating this phenomenon among these three groups vary widely.

The comparative resistance to disinfectants among Gram-negative bacteria varies greatly. A good example of this is the study of Favero and Drake (1966). In 1966, they first applied the term "naturally occurring" to certain Gram-negative bacteria with the potential for rapid growth in water. They observed that *Pseudomonas alcaligenes*, a common bacterial contaminant in iodinated swimming pools, could grow well in swimming-pool waters that had been sterilized by membrane filters and rendered free of iodine or chlorine. Starting with contaminated swimming-pool water that contained a variety of bacteria, they isolated a pure culture of *P. alcaligenes* by an extinction-dilution technique in which filter-sterilized swimming-pool water was used as the diluent and growth medium. Since these cells had been isolated in pure culture without exposure to conventional laboratory culture media, they were referred to as "naturally occurring" *P. alcaligenes*. Subsequent tests showed that these naturally occurring cells were significantly more resistant to free iodine than were cells of the same organism that had been subcultured one time on trypticase soy agar. In fact, standard disinfectant tests using the cells that had been subcultured on an enriched laboratory medium suggested that P. alcaligenes should never be found in pools that had been disinfected even minimally with iodine. This was obviously an erroneous assumption. The discovery that naturally occurring cells were extremely resistant to iodine explained the relatively high concentrations of P. alcaligenes that accumulated in pool water that had been iodinated for several weeks.

Subsequently, Favero et al. (1971, 1975) and Carson et al. (1972) published a series of papers showing that Pseudomonas aeruginosa could grow rapidly in distilled water, which they obtained from hospitals, and could reach high concentrations of cells that remained stable for a long time. Naturally occurring cells that were grown in distilled water reacted quite differently to chemical and physical stresses than did cells grown on standard laboratory culture media. For example, naturally occurring cells of P. aeruginosa were significantly more resistant to chlorine, quaternary ammonium compounds, and alkaline glutaraldehyde than were subcultured cells.

In halogen-disinfected waters, naturally occurring bacteria can be from one to two orders of magnitude more resistant to the disinfectant than cells of the same organism that had been subcultured on conventional laboratory culture media. Since standard disinfectant testing necessarily employs subcultured and washed bacterial cells, a false sense of confidence may be created if these data are used as an absolute criterion for the dilution of a disinfectant. These results could explain the frequent discrepancies between tests that are performed under laboratory conditions and those that are performed under field conditions.

If bacteria could be used in their naturally occurring state, one might explore the possibility of bridging the gaps between laboratory and field conditions by using this experimental system. The ability of some Gram-negative bacteria to grow in water makes it possible to produce and control large numbers of cells for such studies.

More difficult to answer is the more basic question of why naturally occurring cells of Gram-negative water bacteria become more sensitive to disinfectants when grown in a rich medium than the same strain when grown in water . One would expect the reverse to occur. Milbauer and Grossowicz (1959) showed that cells of E. coli were much more sensitive to chlorine when grown on a medium of glucose mineral salts than when grown on nutrient agar. Since Favero and Drake (1966) reported that filter-sterilized dehalogenated swimming-pool water could be considered a minimal medium, one would expect that P. alcaligenes cells that were grown in this environment would be less resistant to iodine than those grown in trypticase soy broth. This phenomenon has not been explained. Evidently it is not primarily a genetic response since the extreme difference in iodine resistance occurs with one subpassage on trypticase soy agar.

Over the years various investigators have tried without success to "train" bacteria to become more resistant to chlorine and/or iodine (Krusé, 1969; Favero, 1961; Favero *et al.*, 1964). This failure is not surprising because if halogens are truly a general cytoplasmic poison that affects primarily the sulfhydryl groups of enzymes (see CHLORINE: Mechanism of Action), it would be very difficult for an organism to modify its physiology to the extent that it becomes resistant, very unlike the situation with antibiotics and bacteria. Consequently, the extreme resistance or differing resistances of naturally occurring bacteria can be attributed only to "environmental" factors and, perhaps, to the different compositions of cell walls and membranes. However, there have been no data to substantiate this hypothesis.

Despite the questions that have been raised by differences in the behavior of organisms under both laboratory and field conditions, valuable comparative information can be obtained from studies of disinfectants that are conducted in similar laboratory systems.

CHLORINE AND CHLORAMINES

Chlorine is a strong oxidizing disinfectant that has been used to treat drinking water supplies for over 60 years. The gas was named "chlorine" after the Greek word for green, "chloros", because of its characteristic color. About 1800 chlorine gas was used as a general disinfectant in both France and England. In the United States, electrolytically produced chlorine was first used directly for water disinfection for only a week or two in 1896 at the Louisville Experimental Station in Kentucky. The first continuous municipal application of chlorine (as sodium hypochlorite [NaOCl]) to water in the United States occurred in 1908 at Jersey City, New Jersey. This was followed in 1912 by the first full-scale use of liquid chlorine for water disinfection at Niagara Falls, New York, where solution-feed equipment was used. This use of chlorine successfully eliminated recurring outbreaks of typhoid fever. In 1913, improved solution-feed equipment was developed to measure chlorine gas, dissolve it in water, and apply the solution to the water supply. This equipment was first installed at Boonton, New Jersey, where it replaced the use of sodium hypochlorite. Other equally significant historical occurrences (Laubush, 1971; White, 1972) led to the eventual addition of chlorine to drinking water in most of the United States for disinfection to destroy or inactivate microorganisms of pathogenic importance (see Drinking Water and Health, NAS, 1977).

Chemistry of Chlorine in Water

Chlorine has an atomic number of 17, a melting point of -102°C, a boiling point of -35°C, and an oxidation potential of -1.36 volts at 25°C (i.e., $2\ Cl^- \rightleftharpoons Cl_2 + 2\ e^-$). It is a green-yellow gas at room temperature.

When chlorine is added to water, the following chemical reactions occur:

$$Cl_2 + H_2O \rightleftharpoons HOCl + H^+ + Cl^- \qquad (1)$$

$$HOCl \rightleftharpoons H^+ + OCl^- \qquad (2)$$

Extremely little molecular chlorine (Cl_2) is present at pH values greater than pH 3.0 and total chlorine concentrations of less than $\sim$1,000 mg/l (White, 1972). The hypochlorous acid (HOCl) that is produced further ionizes to form hypochlorite ion (OCl^-) and hydrogen ion (H^+) (Reaction 2). The dissociation of hypochlorous acid is dependent chiefly upon pH and, to a much lesser extent, temperature, with almost 100% hypochlorous acid present at pH 5, and almost 100% hypochlorite ion present at pH 10 (Figure 1). Free available chlorine refers to the concentration of hypochlorous acid and hypochlorite ion, as well as any molecular chlorine existing in a chlorinated water.

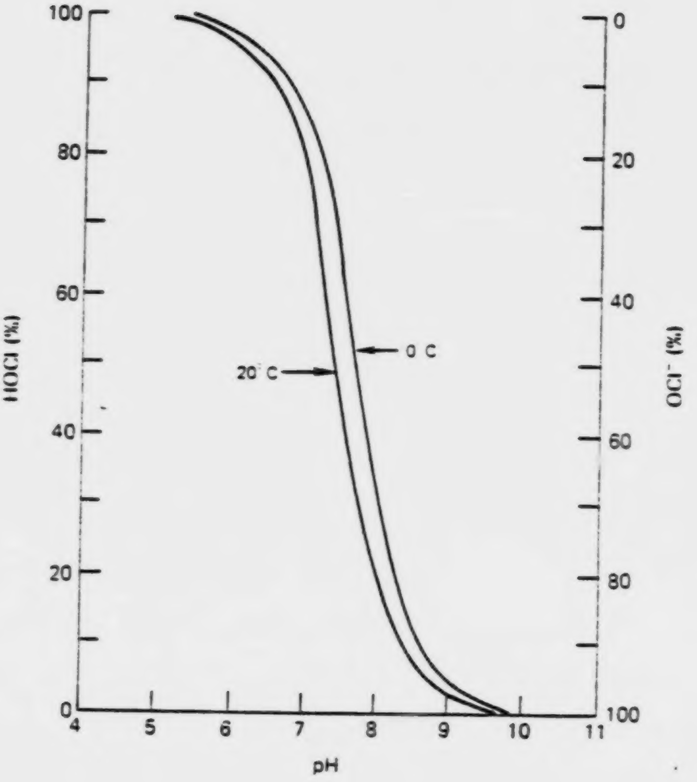

FIGURE 1

Effect of pH on quantities of hypochlorous acid (HOCl) and hypochlorite ion (OCl^-) that are present in water. From Fair *et al.*, 1948.

Chloramine Formation: Inorganic Chloramines

During chlorination of a water supply for disinfection, chlorine will react with any ammonia (NH_3) in the water to form inorganic chloramines. Furthermore, ammonia is sometimes deliberately added to chlorinated public water supplies to provide a combined available chlorine residual, i.e., inorganic chloramines. Chlorine will also react with organic amines. The organic chloramines that are produced (see below) are considered encompassed in the term 'combined available chlorine'.

Although inorganic chloramines are less effective oxidizing and disinfecting agents than hypochlorous acid and hypochlorite ion, they are more stable. Consequently, they will produce a residual in water which will persist for a longer time (Symons et al., 1977).

Inorganic chloramines are formed when hypochlorous acid reacts with ammonia:

$$NH_3 + HOCl \rightleftharpoons NH_2Cl + H_2O \qquad (3)$$

$$NH_2Cl + HOCl \rightleftharpoons NHCl_2 + H_2O \qquad (4)$$

The chloramine that is formed in the reaction depends upon the ratio of ammonia to hypochlorous acid and the pH of the system. Dichloramine ($NHCl_2$) is the predominant form of chloramine at a 1:1 molar ratio of ammonia to chlorine at pH values of 5 and below, whereas at pH values of 9 and above, monochloramine (NH_2Cl) predominates. Figure 2 shows the proportions of monochloramine and dichloramine formed for pH

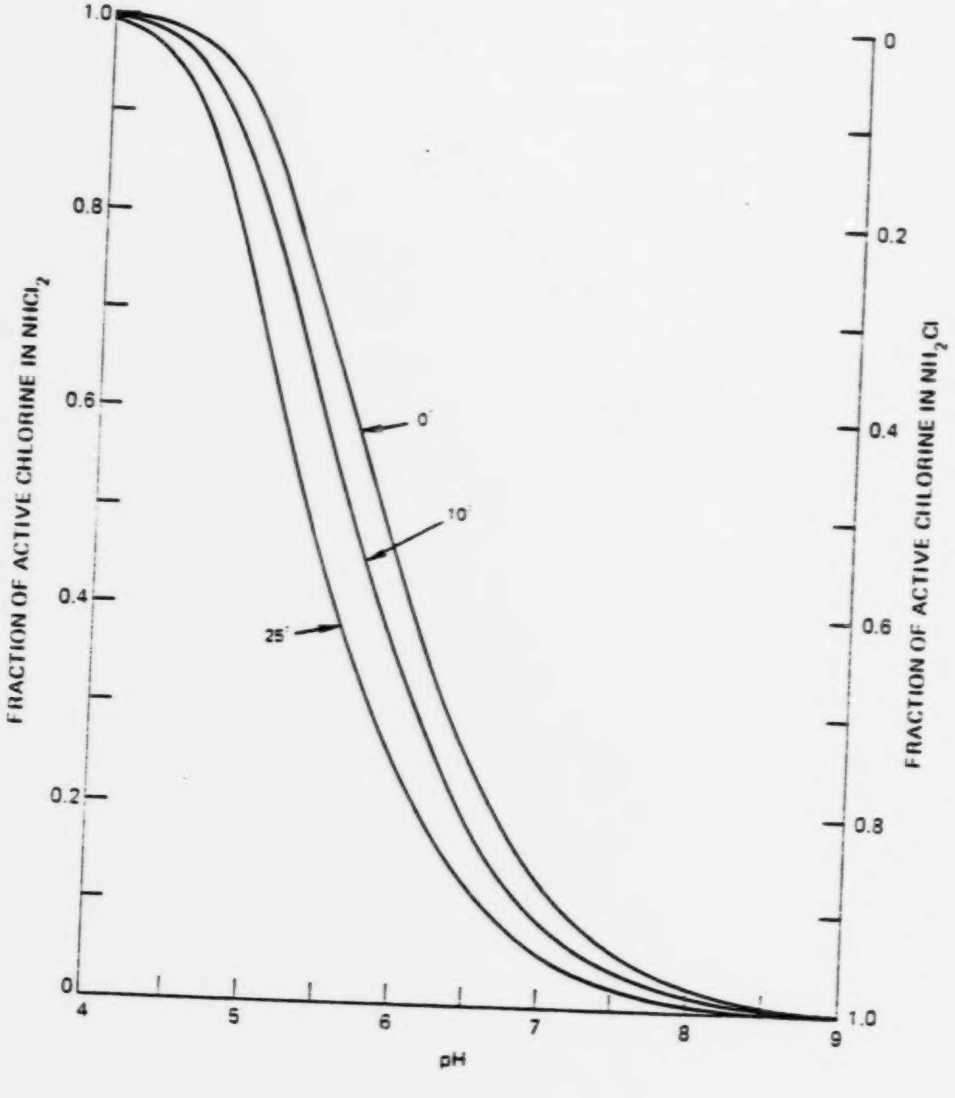

FIGURE 2

Proportions of mono- and dichloramine (NH_2Cl and $HHCl_2$)
in water chlorination with equimolar
concentrations of chlorine and ammonia.
Data from J.C. Morris, personal communication.

values of 4 to 9 and temperatures of 0°C, 10°C and 25°C. (Morris, 1978, personal communication.)

Organic Chloramines

Chlorine is also known to combine slowly with organic or albumenoid nitrogen (amines) to form organic chloramines (Taras, 1953):

$$R\text{-}NH_2 + HOCl \rightleftharpoons R\text{-}NHCl + HOH \qquad (5)$$

Although the reaction between organic amines and chlorine is generally considered to be slow, organic chloramines may be formed, thereby producing a stable combined available chlorine residual after many hours of contact. It is generally accepted that most organic chloramines have little disinfecting capability, i.e., less than the inorganic chloramines (Feng, 1966; Nusbaum, 1952).

Breakpoint Chlorination

Hypochlorous acid and other chlorine compounds having disinfecting ability by virtue of their being oxidizing agents will oxidize sulfites (SO_3^{2-}), sulfides ($S^{=}$), and ferrous (Fe^{2+}) or manganous (Mn^{2+}) ions. The disinfecting species are reduced, and the products have no disinfecting activity. All of the interfering compounds that destroy the disinfecting ability of the added chlorine exert a "chlorine demand," which may be defined as the difference between the amount of chlorine applied and the quantity of free or combined available chlorine residual measured in the water at the end of a specified contact period. When chlorine is added to water with no chlorine demand, a linear relationship is established between the chlorine dosage and the free chlorine residual (Figure 3). However, when increasing amounts of chlorine are added to water containing reducing agents and ammonia, the so-called breakpoint phenomenon occurs. The breakpoint is that dosage of chlorine which produces the first detectable amount of free available chlorine residual.

When chlorine is added to water, it reacts with any reducing agents and ammonia that are present. It is believed that chlorine reacts first with the reducing agents. Since the chlorine is destroyed, no measurable residual is produced. Following the oxidation of these reducing agents, e.g., sulfides, sulfites, nitrites (NO_2^-) and ferrous ions, the chlorine will react with ammonia to form inorganic chloramines. The quantity of monochloramine and dichloroamine that is formed is determined primarily by the pH of the water and the ratio of chlorine to ammonia. When the ratio by weight is less than 5:1, or the molar ratio is less than 1:1, and the pH is in the range of 6.5 to 8.5, the combined available chlorine residual is probably due primarily to monochloramine (Reaction 3). With additional chlorine, the ratio of chlorine to ammonia changes with the result that the monochloramines are converted to dichloramines (Reaction 4). When all of the ammonia has been reacted, a free available chlorine residual

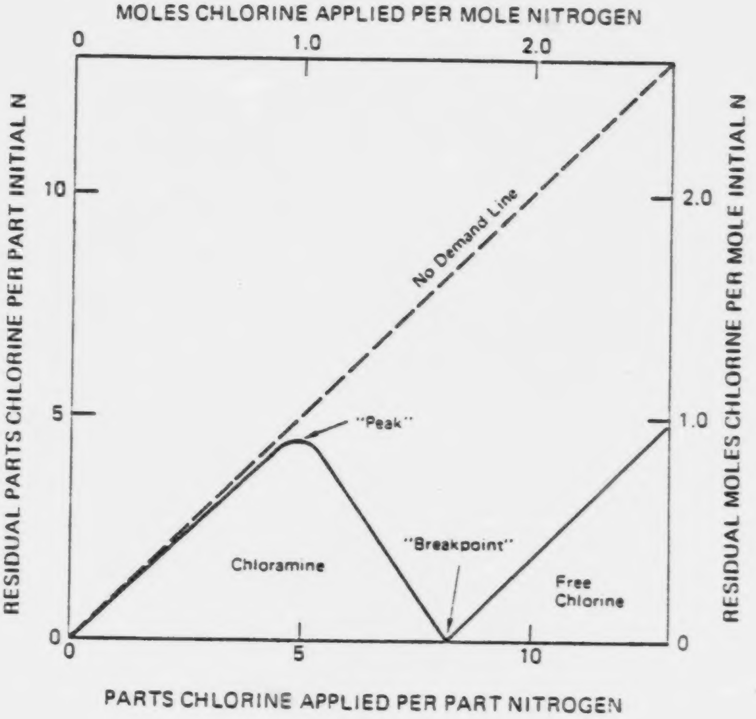

FIGURE 3

Diagrammatic representation of
completed breakpoint reaction.
From Morris, 1970, with permission.

begins to develop. As the concentration increases, the previously formed chloramines are oxidized to nitrous oxide (N_2O), nitrogen trichloride (NCl_3) and nitrogen (N_2). The reactions leading to the formation of these oxidized forms of nitrogen destroy the combined available chlorine residual so that the measurable residual in the water actually decreases. Upon completion of the oxidation of all the chloramines, the addition of more chlorine creates the breakpoint phenomenon. At the breakpoint dosage, some resistant chloramines may still be present, but at such small concentrations that they are unimportant.

As pointed out by Morris (1970), the occurrence of reactions giving rise to the "breakpoint" is most rapid in the pH range 7.0 to 7.5. At greater and lesser pH values it becomes slower and less distinct; e.g., at pH's < 6 or > 9 the concept of "breakpoint" is not significant. In the pH range 7 to 7.5 the "breakpoint" is about half developed within 10 minutes at 15°C to 20°C and is then substantially completed within about 2 hours.

Analytical Methods and Their Evaluation

Standard Methods (1976) lists six acceptable methods for the determination of chlorine residuals in natural and treated waters: iodometric methods, amperometric titration, the stabilized neutral orthotolidine (SNORT) method, the ferrous diethyl-p-phenylenediamine (DPD) method, the DPD colorimetric method, and the leuco crystal violet (LCV) method.

The amperometric, LCV, DPD, and SNORT methods are unaffected by dichloramine concentrations in the range of 0 to 9 mg/l (as Cl_2) in the determination of free chlorine. If nitrogen trichloride is present, it reacts partially as free available chlorine in the amperometric, DPD, and SNORT methods. Nitrogen trichloride does not interfere with the LCV procedure for free chlorine. The sample color and turbidity may interfere with all colorimetric procedures. Thus, a compensation must be made. Also, organic contaminants in the sample may produce a false free chlorine reading in most colorimetric methods.

Standards Methods (1976) contains data on the precision and accuracy of the methods used in the measurement of chlorine. These data were obtained from participating laboratories by the Analytical Reference Service (1969, 1971), which then operated in an agency that preceded the Environmental Protection Agency. However, as noted in _Standard Methods_ (1976), these results are valuable only for comparison of the methods tested, and many factors, such as analytical skill, recognition of known interferences, and inherent limitations, determine the reliability of any given method. Moreover, some oxidizing agents, including free halogens other than chlorine, will appear quantitatively as free chlorine. This is also true of chlorine dioxide. Also, some nitrogen trichloride may be measured as free chlorine. The actions of interfering substances should be familiar to the analyst because they affect a particular method. Although orthotolidine (i.e., orthotolidine and orthotolidine arsenite) methods have been widely used in many disinfection studies, they are omitted from the 14th edition of Standard Methods (1976) primarily because of their inaccuracy and high overall (average) total error in comparison with other available methods.

Research studies on disinfection are restricted by the limitations that are inherent in the methods themselves or by poor selection of methods by the investigator. The chemical conditions of the test water have not always been well defined. The types of titratable chlorine, i.e., free (hypochlorous acid or hypochlorite ion) or combined (mono- or dichloramine) in the chlorinated water, have not always been differentiated, and the rates of microbial destruction or inactivation have not always been studied in experimental systems with little or no chlorine demand. In fact, reports prior to the 1940's have been especially difficult to interpret because reliable test methods for distinguishing between free and combined chlorine, between hypochlorous acid and hypochlorite ion, and between mono- and dichloramine in solution were not developed until the 1950's. For example, many earlier researchers claimed to have tested mono- and dichloramine by controlling the pH and the ratio of chlorine to nitrogen. They used methods such as the orthotolidine or thiosulfate titrations to determine total chlorine residual. Much of this early work is now questionable since it was not possible to detect free chlorine contamination in their chloramine solutions or the quantitative ratios between the mono- and dichloramine tested. In addition, these earlier studies had high chlorine demand in the test systems.

Some more contemporary studies have lacked quantitated information on chlorine residual and/or types of chlorine present in the test systems.

Biocidal Activity

In the absence of reducing agents, inorganic ammonia, and organic amines, the addition of chlorine to municipal water supplies will result in free available residual chlorine, represented by the hypochlorous acid or hypochlorite ion. The pH determines the relative amounts of each species. However, inorganic chloramines will be formed if the background level of ammonia in the water supply is significant or if ammonia is intentionally added during treatment. If such is the case, monochloramine would predominate due to the alkaline pH of most finished water (see Figure 2).

In 1966, Feng proposed that the active forms of chlorine would exhibit disinfection properties in the following descending order:

$$Cl_2 > HOCl > OCl^- > NHCl_2 > NH_2Cl > R\text{-}NHCl$$

Butterfield *et al.* (1943) published the first treatise on the use of chlorine demand-free water for studies of water disinfection. They proposed that to study the disinfectant capacity of any chlorine species, the test medium must meet certain exacting criteria. It must be non-toxic to bacteria except for the variables under study such as chlorine and pH; well buffered at the desired pH; free of all ammonia and organic matter capable of forming chlorine-addition products; free of background chlorine; and of such a nature that calculated additions of chlorine are recoverable after 5 minutes without a loss in residual and that free chlorine must still be present several hours after contact.

Most studies of combined chlorine have dealt with poorly defined mixtures of mono- and dichloramine. Also, test conditions have often been inadequately defined, poorly controlled, or both.

Efficacy Against Bacteria

Free Chlorine ($HOCl$ and OCl^-): Butterfield *et al.* (1943) studied percentages of inactivation as functions of time for *E. coli*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Shigella dysenteriae*. They used different levels of free chlorine at pH values ranging from 7.0 to 10.7 and two temperature ranges -- 2°C to 5°C and 20°C to 25°C. Their work is of great importance since very few other studies have been conducted which dealt with the action of disinfectants on pathogens. Generally, they found that the primary factors governing the bactericidal efficacy of free available chlorine and combined available chlorine were:

- -- the time of contact between the bacteria and the bactericidal agent, i.e., the longer the time, the more effective the chlorine disinfection process;
- -- the temperature of the water in which contact is made, i.e., the lower the temperature, the less effective the chlorine disinfecting activity; and
- -- the pH of the water in which contact is made, i.e., the higher the pH, the less effective chlorination.

Thus, the test bacteria will be killed more rapidly at lower pH values and at higher temperatures. Since hypochlorous acid would predominate at lower pH's (Figure 1), the data of Butterfield et al. show that it is a better bactericide than the hypochlorite ion. For example, to produce a 100% inactivation of an initial inoculum of 8 x 10^5 E. coli in 400 ml of sterile chlorine demand-free water (2,000/ml) at 20°C-25°C with a chlorine level of 0.046 to 0.055 mg/l, 1.0 minute was required at pH 7.0, but at pH 8.5, 9.8, and 10.7, between 20 and 60 minutes of exposure were needed. At higher concentrations of chlorine, i.e., from 0.1 to 0.29 mg/l, exposure of 1.0 minute was required at pH 7.0, 10 minutes at pH 8.5, 20 minutes at 9.8, and 60 minutes at 10.7. A similar pH effect was noted for S. typhi.

Unfortunately, Butterfield _et_ _al_. (1943) lifted their cells from agar slants but failed to wash them in demand-free water. The cells probably carried trace amounts of albumenoid nitrogen from the slants to the test flasks thereby creating the small chlorine demand that the investigators had tried so carefully to avoid. The effect of such a trace amount of chlorine demand would be most apparent in test solutions with very low chlorine levels. In studies using approximately 0.1 mg/l or less free chlorine at decreasing pH values, Butterfield _et_ _al_. (1943) observed that the disinfection of the organisms required a very long time. This might indicate interference at the low levels due to the formation of combined chlorine.

Under very exact controlled test conditions of pH and temperature and using chlorine demand-free buffer systems, Scarpino _et_ _al_. (1972) observed that at 5°C, _E_. _coli_ was 99% destroyed by 1.0 mg/l hypochlorous acid at pH 6 in less than 10 seconds and at pH 10 by 1.0 mg/l hypochlorite ion in about 50 seconds. Their studies, which totally eliminated any form of combined chlorine from the test solutions, indicated that hypochlorous acid was approximately 50 times more effective than the hypochlorite ion as a bactericide. Fair _et_ _al_. (1948) and Berg (1966) analyzed the data of Butterfield _et_ _al_. (1943) on the destruction of _E_. _coli_. They reported that hypochlorous acid was 70-80 times as bactericidal as hypochlorite ion.

Engelbrecht *et al*. (1975) investigated new microbial indicators of disinfection efficiency. In their chlorination studies, they noted the following decreasing order of resistance to free chlorine at pH values of 6, 7, and 10 and at 5°C and 20°C: acid-fast bacteria > yeasts > poliovirus > *Salmonella typhimurium* > *E. coli*.

Monochloramine (NH_2Cl): In 1948, Butterfield summarized previous results on the bactericidal properties of chloramines (and free chlorine) in water at pH values ranging from 6.5 to 10.7 and in two temperature ranges-- 2°C to 5°C and 20°C to 25°C. The test bacteria included strains of *Escherichia coli*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Shigella dysenteriae*. Although he admitted that adequate tests for separate determination of free and combined chlorine forms were not used in these studies, the solutions were vigorously prepared to insure the exclusions of free chlorine. Chloramines were determined using orthotolidine; readings made after 10 to 30 seconds at 20°C gave free chlorine levels, and those after standing for 10 minutes at 20°C were recorded as total residual chlorine. Since no free chlorine was reported (and should not have been found, according to the authors), the 10-minute readings of total residual chlorine were also those of total chloramine levels. No distinction could be made between monochloramine and dichloramine. However, he estimated that at pH 6.5, 7.0, 7.8, 8.5, 9.5, and 10.5 the chloramines were present as monochloramine at 35%, 51%, 84%, 98%, 100%, and 100%, respectively. The balance was believed to be dichloramine.

Butterfield and his associates (Butterfield *et al.*, 1946; Butterfield, 1948) found that chloramine disinfection was always slower than that for free chlorine. For example, in order to achieve a 100% inactivation of the initial number of bacteria tested after 60 minutes of contact time at 20°C, 0.6 mg/l chloramine was required at pH 7.0, and 1.2 mg/l at pH 8.5. At 4°C, a 100% inactivation required 1.5 mg/l chloramine at pH 7.0, and 1.8 mg/l at pH 8.5. However, only 0.03 to 0.06 mg/l free chlorine was needed at pH ranges of 7.0 and 8.5 at either 4°C or 22°C to achieve 100% inactivation in 20 minutes.

The bactericidal effects of monochloramine alone were confirmed by Siders *et al.* in 1973. They found that at 15°C *E. coli* was 99% destroyed in approximately 20 minutes using 1.0 ppm monochloramine in pH 9 borate buffer. Since *E. coli* was less resistant to monochloramine than were the animal viruses tested, Siders *et al.* questioned the validity of using *E. coli* as an indicator organism for measuring the viral quality of a chlorinated water supply. Chang (1971) had previously calculated that 4 mg/l monochloramine would be needed to give 99.999% reduction of *E. coli* bacterium in 10 minutes at 25°C.

Dichloramine ($NHCl_2$): Chang (1971) calculated that 1.2 mg/l dichloramine would be needed to give 99.999% reduction of enteric bacteria in 10 minutes at 25°C.

In carefully conceived studies, Esposito et al. (1974) examined the destruction rates of test organisms in contact with dichloramine in demand-free phthalate buffer at pH 4.5 and 15°C. Figure 4 shows the comparisons that were made among enteroviruses (poliovirus 1 and coxsackievirus A9), the bacteriophage ΦX-174, and E. coli (ATCC 11229).

From a review of the literature and an analysis of the data, Chang (1971) calculated that the relative bactericidal efficiency of dichloramine to monochloramine was 3.3:1. However, Esposito (1974) and Esposito et al., (1974) showed experimentally that Chang's estimate was conservative. They found that dichloramine was 35 times more bactericidal than was monochloramine, not 3.3. At 15°C, poliovirus 1 was 17 times more resistant than coxsackievirus A9, 83 times more resistant than ΦX-174, and 1,700 times more resistant than E. coli to dichloramine (Figure 4). They observed that dichloramine was a better bactericide than monochloramine.

Organic Chloramines: These chlorine derivatives exhibit some bactericidal activity, but markedly less than either free chlorine or the inorganic chloramines (Feng, 1966; Nusbaum, 1952).

In summary, the bactericidal efficiency of hypochlorous acid, the hypochlorite ion, monochloramine, and dichloramine have been accurately defined in recent years by investigators using rigidly controlled test conditions. The

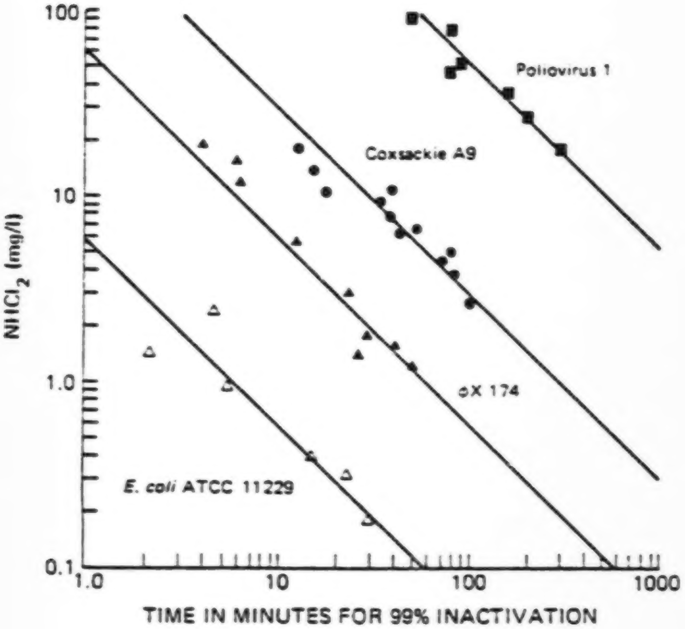

FIGURE 4

Inactivation of various microorganisms with dichloramine ($NHCl_2$) at pH 4.5 and 15°C. From Esposito, 1974.

order of disinfection efficiency presented by Feng (1966) has been confirmed. Comparative c.t values are shown in Table 3 in the Summary of the section on chlorine.

Efficacy Against Viruses

In reviewing disinfection of enteroviruses in water, Clarke and Chang (1959) excluded all studies on the inactivation of viruses by chlorine which were conducted before 1946. Their justification for this exclusion was the failure of these studies to differentiate between free and combined chlorine. Furthermore, they attributed the irregular virucidal results of some studies to the use of animal inoculation methods for assaying virus concentrations. For these same reasons, those studies have been omitted in this report. The advent of viral propogation techniques using tissue cultures (Enders *et al*., 1949) enabled research of a more exacting nature to be performed, resulting in more precise virus inactivation data.

Free Chlorine (HOCl and OCl^-): Generally, enteroviruses are more resistant to free chlorine than are the enteric bacteria (Chang, 1971; Clarke and Kabler, 1954; Scarpino *et al*., 1972). For example, in what was probably the first well-defined study, Clarke and Kabler (1954) used purified coxsackievirus A2 to investigate viral inactivation in water by free chlorine. They carefully controlled their free chlorine residuals with a modified form of the orthotolidine test to determine total chlorine and an orthotolidine-arsenite method for free chlorine. (Combined chlorine was then calculated as the difference between "total" and "free" chlorine readings.) They measured virus recoveries by using suckling mice and the LD_{50} quantitation procedure. Their results indicated that inactivation times for the virus increased with increasing pH (6.9 to 9.0), decreasing temperatures (27°C-29°C to 3°C-6°C), and decreasing total chlorine concentration. They estimated that approximately 7 to 46 times as much free chlorine was required to obtain comparable inactivation of coxsackievirus A2 as was required for a suspension of _E_. _coli_ cells (Butterfield _et_ _al_., 1943). For instance, Butterfield _et_ _al_. (1943) found that at pH 7.0 and at 2°C to 5°C, 99.9% of _E_. _coli_ cells were inactivated in 5 minutes with 0.03 mg/l of free chlorine. At approximately the same pH and temperature ranges, Clarke and Kabler (1954) observed 99.6% inactivation of coxsackievirus A2 in 5 minutes with 1.4 mg/l of free chlorine; i.e., 46 times as much free chlorine as that required to inactivate _E_. _coli_ cells. At a pH of 8.5 at 25°C, 99.9% of _E_. _coli_ cells were inactivated in 3 minutes with 0.14 mg/l of free chlorine (Butterfield _et_ _al_., 1943), while at a pH of 9.0 at 27°C to 29°C, 99.6% of the virus was inactivated in 3 minutes by 1.0 mg/l of free chlorine. Thus, Clarke and Kabler's work showed that 7 times as much free chlorine was required to inactivate the test coxsackievirus compared to the time necessary to kill the bacterium E. coli. In a subsequent study, Clarke et al. (1956) found that adenovirus type 3, E. coli, and Salmonella typhi were all inactivated or destroyed at approximately the same concentration of free chlorine.

In 1958, Weidenkopf reported his studies on the rate of inactivation of poliovirus 1 as a function of free available chlorine (HOCl and OCl^-) and pH at 0°C. His results showed that 99% inactivation of poliovirus 1 was obtained with 0.10 mg/l free chlorine in 10 minutes at pH 6.0 and of 0°C. At pH 6, most of the free chlorine should have been present as hypochlorous acid. Increasing the pH to 7.0 increased the time required for the same degree of inactivation by approximately 50%. At that pH, the free chlorine should have been a mixture containing predominantly hypochlorous acid and significant levels of hypochlorite ion. Both Weidenkopf (1958) and Clarke et al. (1956) indicated that an increase in pH (from 7.0 to 8.5, Weidenkopf; from 8.8 to 9.0, Clarke et al.) increased the inactivation time about sixfold. At these pH's, the free chlorine should have been present as mixtures of both hypochlorous acid and hypochlorite ion, but predominantly as the ion.

After comparing these studies, Clarke et al. (1964) concluded that at 0°C to 6°C poliovirus 1 and coxsackievirus A2 were considerably more resistant to hypochlorous acid than was E. coli, while adenovirus type 3 was more sensitive (Figure 5). For 99% destruction of E. coli, a 99-second contact time was required when the system was dosed with 0.1 mg/l free chlorine as hypochlorous acid. The same percentage of the adenovirus was inactivated in approximately one-third of that time by the same concentration of hypochlorous acid. Under the same conditions, 8.5 minutes was required to inactivate 99% of the poliovirus, i.e., about five times the contact time required for _E_. _coli_. Coxsackievirus required a contact time for 99% inactivation in excess of 40 minutes, more than 24 times that required for _E_. _coli_.

Clarke and Kabler (1954) and Clarke _et_ _al_. (1956) reported that the time required for inactivation of coxsackievirus A2 and adenovirus increased with increasing pH, decreasing total chlorine concentrations, and decreasing temperatures. Clarke and Chang (1959) concluded from data in the literature that a 10°C increase in temperature increased the rate of virus inactivation by a factor of 2 to 3.

Kelly and Sanderson (1958) reported that each of six enteric viruses possessed a different sensitivity to chlorine. Their results suggested that the inactivation of enteric viruses in water at pH 7.0 and 25°C required a minimum free residual chlorine concentration of 0.3 mg/l with a contact time of at least 30 minutes. With combined chlorine in water, a concentration of at least 9.0 mg/l was necessary for a 99.7% inactivation of poliovirus at 25°C and a pH of 7.0. Poliovirus 1 (strain MK 500) was the most

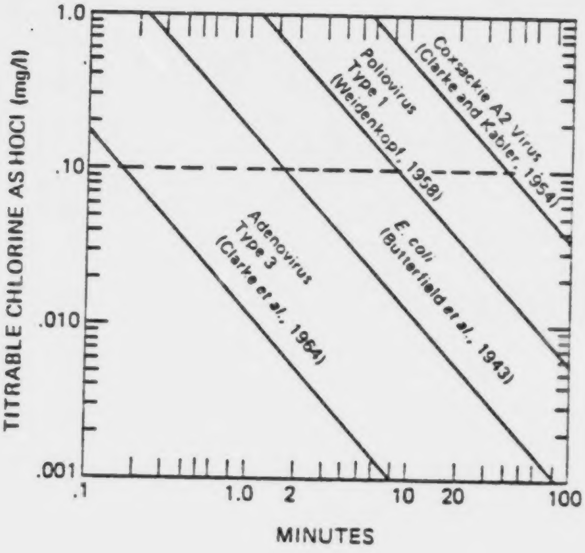

FIGURE 5

Concentration-time relationship for 99% destruction of E. coli and several viruses by hypochlorous acid at 0-6°C. From Clarke et al., 1964.

resistant strain tested and coxsackievirus B5 the most sensitive. Poliovirus 1 (Mahoney strain), poliovirus 2, coxsackievirus B1, and poliovirus 3 were intermediate in resistance. The virucidal efficiency of hypochlorous acid was more than 50 times greater than that of the chloramines (Kelley and Sanderson, 1958, 1960).

Liu et al. (1971) studied the manner in which 20 strains of human enteric viruses responded to free chlorine. They used Potomac River water that had been partially treated by coagulation with alum and filtration through sand. Chlorine was added to the water at one dosage, 0.5 mg/l. The final pH was 7.8. They stored the sample at 2°C. There was a wide range of resistance to chlorine by the viruses. The most sensitive virus was reovirus type 1, which required 2.7 minutes for inactiving 4 logs (99.99%) of the virus with 0.5 mg/l of free chlorine. The most resistant, as judged by extrapolating the experimental data, was poliovirus 2, which required 40 minutes for the same degree of inactivation. Using actual experimental data, the most resistant virus was echovirus 12, which required a contact time of greater than 60 minutes for 99.99% inactivation. Liu et al. (1971) concluded from their extrapolated values that the reoviruses were the least resistant to chlorine treatment; that both adenoviruses and echoviruses were less resistant; and that the polioviruses and coxsackieviruses were the most resistant. However, assuming a 20-minute contact time, most of the viruses tested at pH 7.8 and 2°C would have been

99.99% inactivated with a free chlorine residual of 0.5 mg/l.

Using six of the same virus strains studied by Liu et al. (1971), Engelbrecht et al. (1978) investigated the effect of pH on the kinetics of chlorine inactivation at 5.0 ± 0.2°C. The suspending medium was buffered, chlorine demand-free, distilled-deionized water. Each virus stock was also prepared so as to be chlorine demand-free. Table 1 summarizes the results, giving the chlorine levels used and the time required for two logs (99%) inactivation of the viruses at pH 6.0 and 7.8 in phosphate buffer and at pH 10.0 in borate buffer. Because of the use of two different buffer systems, i.e., at pH 6.0 and 10.0, virus inactivation was determined at pH 7.8 with each of the two buffer systems. The kinetics of inactivation of poliovirus 1 at pH 7.8, using both the phosphate and borate buffer, were the same. The results shown in Table 1 indicate that there is a significant difference in the time required for two logs inactivation for the various viruses at pH 6.0 and 10.0. In every case, the rate of inactivation at pH 10.0 was significantly less than at pH 6.0. The rank ordering in Table 1 shows that there is also a wide range of sensitivity of related viruses to chlorine disinfection. For example, at pH 10.0, coxsackie B5 was 40 times more resistant than coxsackie A9. There are several cases in which the relative sensitivity to chlorine was altered (rank ordering) between pH 6.0 and 10.0, suggesting important effects of pH on the virion as well as on the chlorine species, i.e., hypochlorous acid vs. hypochlorite ion. This observation can be seen more clearly in Table 2 in which the time required for two logs of inactivation of the various viruses and the ratio of inactivation times at pH 6.0 and 10.0 are compared. Even at pH 7.8, differences in relative sensitivity appear when ranked and compared to results at pH 6.0 or 10.0 (Table 1).

Combined Chlorine (NH_2Cl and $NHCl_2$): Viral inactivation rates with chloramines have been found to be much slower than with free chlorine. For example, Kelly and Sanderson (1958) studied the effects of chlorine on several enteric viruses. They reported that at pH 7 at 25°C-28°C, 0.2-0.3 mg/l free chlorine inactivated 99.9% of all test viruses in 8 minutes. At the same temperature and pH, combined chlorine at 0.7 mg/l and at least 4 hours of contact time were needed to achieve 99.7% inactivation of the test viruses.

Although most viral inactivation studies with chloramines have not differentiated between mono- and dichloramine (Kelly and Sanderson, 1958; Lothrop and Sproul, 1969), Kelly and Sanderson (1958, 1960) noted that viral inactivation by chloramines proceeds more rapidly at pH 6-7 than at pH 8-10. This tendency indicates that dichloramine may be more virucidal than monochloramine since its proportion increases with increased hydrogen ion concentration.

TABLE 1

Time Required for 99% Inactivation by Free Residual Chlorine At 5.0°C ± 0.2°C[a]

pH	Concentration of Free Chlorine, mg/1[b]	Virus Strain	Minutes for 99% inactivation	Rank Ordering
6.00	0.46-0.49	Coxsackie A9 (Griggs)	0.3	1
6.00	0.48-0.49	Echo 1 (Farouk)	0.5	2
6.00-6.02	0.48-0.51	Polio 2 (Lansing)	1.2	3
6.00-6.03	0.39-0.49	Echo 5 (Noyce)	1.3	4
6.00	0.47-0.49	Polio 1 (Mahoney)	2.1	5
6.00-6.06	0.51-0.52	Coxsackie B5 (Faulkner)	3.4	6
		Coxsackie A9 (Griggs)	ND	
7.81-7.82	0.47-0.49	Echo 1 (Farouk)	1.2	1
		Polio 2 (Lansing)	ND[c]	
7.79-7.83	0.48-0.52	Echo 5 (Noyce)	1.8	3
7.80-7.84	0.46-0.51	Polio 1 (Mahoney)	1.3	2
7.81-7.82	0.48-0.50	Coxsackie B5 (Faulkner)	4.5	4
10.00-10.01	0.48-0.50	Coxsackie A9 (Griggs)	1.5	1
10.00-10.40	0.49-0.51	Echo 1 (Farouk)	96.0	6
9.89-10.03	0.48-0.50	Polio 2 (Lansing)	64.0	4
9.97-10.02	0.49-0.51	Echo 5 (Noyce)	27.0	3
9.99-10.40	0.50-0.52	Polio 1 (Mahoney)	21.0	2
9.93-10.05	0.50-0.51	Coxsackie B5 (Faulkner)	66.0	5

[a] Data from Engelbrecht *et al.*, 1978.

[b] Range of measured free chlorine residual in the "test" reactor at the termination of each of three separate experiments.

[c] ND = not determined.

TABLE 2

Comparison of Virus Inactivation by Free Residual Chlorine[a] at pH 6.0 and 10.0, and at 5.0°C ± 0.2°C[b]

Virus Strain	Minutes for 99% Inactivation		
	pH 6.0	pH 10.0	Ratio[c]
Coxsackie A9 (Griggs)	0.3	1.5	5
Echo 1 (Farouk)	0.5	96.0	192
Polio 2 (Lansing)	1.2	64.0	53
Echo 5 (Noyce)	1.3	27.0	21
Polio 1 (Mahoney)	2.1	21.0	10
Coxsackie B5 (Faulkner)	3.4	66.0	19

[a] Chlorine values as noted in Table 1 at pH 6.0 and 10.0.

[b] Data from Engelbrecht *et al*., 1978.

[c] $\frac{\text{Time required at pH 10.0}}{\text{Time required at pH 6.0}}$

In 1971, Chang proposed that 5.0 mg/l dichloramine or 20 mg/l monochloramine would be needed to inactivate enteroviruses by 99.99% in 10 minutes at 25°C.

Subsequently, Siders _et al_. (1973) presented evidence of the first comparative studies on viral inactivation due solely to monochloramine. At pH 9 at 15°C, poliovirus 1 (Mahoney) was 10 times more resistant to monochloramine than was _E. coli_. Similarly, coxsackievirus A9 was approximately four times more resistant than _E. coli_. Siders' data can be compared to Chang's theory on the disinfectant capacity of monochloramine. Assume that poliovirus inactivation has a temperature coefficient for a 10°C rise (Q_{10}) of 3. Based on Chang's (1971) calculations that 20 mg/l monochloramine at 25°C would be needed to achieve 99.999% reduction of enterovirus in 10 minutes, one would extrapolate that 3x20 or 60 mg/l monochloramine would be needed at 15°C to achieve an equivalent reduction of poliovirus in 10 minutes. However, examination of Siders' _et al_. data reveals that approximately 18 minutes were needed to achieve only 99% inactivation using 60 ppm monochloramine. Therefore, it appears that Chang slightly overestimated the virucidal capacity of monochloramine.

Efficacy Against Parasites

Some studies, mostly in the field of wastewater treatment, have shown that ova and larvae of the helminth parasites that affect humans and could occur in U.S. water supplies are resistant to current chlorination procedures. They can survive concentrations and exposure periods considerably in excess of those used in the treatment of municipal water supplies. In studies of various free-living nematodes, Chang _et_ _al_. (1960) observed that 2.5 to 3.0 mg/l of free chlorine for a 120-minute contact period and 15 to 45 mg/l of free chlorine for 1 minute were not lethal. Free chlorine residuals as high as 95 to 100 mg/l for 5 minutes killed only 40%-50% of the nematodes. Thus, it may be speculated that all the helminths, including their larvae, may approach the degree of resistance to chlorine that had been demonstrated by the free-living nematodes.

There have been a number of studies on the effectiveness of chlorine in destroying or inactivating cysts of the protozoan parasite, _Entamoeba_ _histolytica_, in water, especially during the early 1940's (Brady _et_ _al_., 1943; Chang, 1944b; Chang and Fair, 1941). Varied results reflect primarily the different experimental conditions and techniques that were used. The presence of organic matter, pH, and temperature, as well as the concentration and form of chlorine and exposure period have been shown to exert an influence on disinfection. However, the consensus is that, compared with bacteria, these cysts are rather resistant to current chlorination procedures, but are much less resistant than helminths.

Brady _et_ _al_. (1943) conducted field-simulated studies with cysts in raw water that had been treated with calcium hypochlorite (CaOCl), resulting in pH levels ranging from approximately 7.5 to 8. They found that at temperatures of 23°C-26°C, exposures of 20 minutes and longer to residuals of 3 to 4 mg/l were required to produce an estimated 99% cyst destruction via a culture technique. Chang (1944), also using a culture technique, studied the cysticidal effectiveness of calcium hypochlorite solution, chloramines, and gaseous chlorine in tap water as well as the effects of pH and organic matter on the biocidal activity. At contact periods of up to 30 minutes, gaseous chlorine was the most powerful, hypochlorite solution slightly less so, and chloramines the least. Increase in pH and organic matter reduced cysticidal efficacy. For comparison with Brady et al. (1943), the "lethal" residual concentration in tap water at 18°C and pH 6.8-7.2 ranged from 2.8 to 3.2 mg/l at 15 minutes, and from 1.8 to 2.2 mg/l at 30 minutes (as estimated from a graph in Chang, 1944b).

Recently, Stringer et al. (1975) reported on comparative studies of the cysticidal efficacy of chlorine, bromine, and iodine as disinfectants. Using chlorine gas bubbled into buffered distilled water as stock, they obtained 99.9% cyst inactivation (as measured by excystment capability) after 15 minutes exposure to 2 mg/l free chlorine in "clean water" at pH 6. However at pH 8 a contact time exceeding 60 minutes was required to achieve 99% mortality. In "secondary treated sewage effluent," Stringer et al. considered 13.7 mg/l chlorine at pH 8 to be ineffectual as a cysticide.

In keeping with these findings, it is unlikely that the chlorine residuals generally maintained in distribution systems provide much protection against *E. histolytica* cysts in the event of contamination because of cross-connections, seepage, etc.

During the past 10 years, a number of outbreaks of waterborne infections from *Giardia lamblia* (another intestinal protozoan) have been reported (NAS, 1977). Most incidents in the United States that were traced to municipal water supplies involved surface water sources where disinfection appeared to be the only treatment. The cysts of this parasite are thought to be as resistant to chlorine as those of *E. hystolytica*, However, there seem to be no studies of the resistance of this parasite to chlorine or other disinfectants.

Mechanism of Action

One of the earliest references to the mechanism of inactivation of microorganisms by chlorine resulted from the work of Chang (1944a, b). While studying the inactivation of *E. histolytica* cysts by chlorine, he observed greater uptake of chlorine and less survival at low pH than at high pH. This observation was associated with the increased inactivation efficiency of the undissociated hypochlorous acid. Supportive evidence for the hypothesis that permeability of the uncharged chlorine species is important in determining sensitivity to chlorine has been provided by

Skvortsova and Lebedeva (1973), Kaminski *et al*. (1976), and Dennis (1977). Chang (1944a) also noted that the inactivation of amoebic cysts was accompanied by microscopic damage to the cell nucleus which was dependent on chlorine penetration.

The importance of penetration and/or damage to the permeability barrier of the cell membrane as a result of exposure to chlorine has been observed by several investigators. In 1945, Rahn suggested that the inactivation of bacteria by chlorine was due to multiple injuries to the cell surface. From their work with bacterial spores, Kulikovsky *et al*. (1975) implicated permeability damage as a mechanism of chlorine inactivation. Studies with *Escherichia coli* have shown that chlorine causes leakage of cytoplasmic material, first protein, then RNA and DNA, into the suspending menstruum. It also inhibits the biochemical activities that are associated with the bacterial cell membrane (Venkobachar, 1975; Venkobachar *et al*., 1977). Friberg (1957) observed that *E. coli* also loses non-dialyzable phosphorus following exposure to chlorine. In a recent study, Haas (1978) demonstrated that chlorine caused certain bacteria and yeast to release organic matter or UV-absorbing material, presumably protein or nucleic acid or their precursors. This investigator also noted that chlorine affected the uptake and retention of potassium by these same microorganisms.

Green and Stumpf (1946) and Knox et al. (1948) indicated that destruction of bacteria by chlorine was caused by an inhibition of the mechanism of glucose oxidation. Specifically, they suggested that chlorine affected the aldolase enzyme of E. coli by oxidizing the sulfhydryl group that is associated with the enzyme. Venkobachar et al. (1977) recently reported that chlorine significantly inhibits both oxygen uptake and oxidative phosphorylation. The latter effect was attributed to inhibition of the respiratory enzyme rather than to a deficiency in phosphate uptake. However, it is unclear whether free or combined chlorine was used in these studies. Haas (1978) also observed chlorine to affect the respiration of bacteria as well as the rate of synthesis of protein and DNA. Others have also noted that chlorine affects the nucleic acids or physically damages DNA (Bocharov, 1970; Fetner, 1962; Bocharov and Kulikovskii, 1971; Rosenkranz, 1973; Shih and Lederberg, 1976a,b).

It appears that chlorine, having penetrated the cell wall, encounters the cell membrane and alters its permeability. Simultaneously or subsequently, the chlorine molecules may enter the cytoplasm and interfere with various enzymatic reactions. It should be noted that permeases and respiratory enzymes are associated with the cytoplasmic membrane of bacteria.

Chang (1971) supported the hypothesis that the rapid destruction of vegetative bacteria by chlorine was due to the extensive destruction of metabolic enzyme systems. He also addressed the subject of virus inactivation, commenting that viruses are generally more resistant to chlorine than bacteria. He associated this observation with the fact that viruses completely lack a metabolic enzyme system. He speculated that inactivation of viruses by chlorine probably result from the denaturation of the capsid protein. Furthermore, since protein denaturation is more difficult to achieve than destruction of enzymatic R-S-H bonds by oxidizing agents, it is understandable why greater levels of chlorine are required to inactivate viruses than bacteria. However, from their experimental work with the bacterial virus f2 Olivieri et al. (1975) concluded that chlorine caused initial lethal damage to the viral genome and that the capsid protein was affected after the virus was inactivated. Dennis (1977) reported that the incorporation of chlorine into the f2 bacterial virus is dependent on pH and that the higher rates of incorporation occur at lower pH values.

There is limited information in the literature on the mechanism of inactivation of microorganisms by chloramines. Nusbaum (1952) proposed that since low levels of inorganic chloramines were effective in inactivating bacteria, the mechanism of action must be essentially the same as that of hypochlorous acid on enzymes. Ingols et al. (1953) showed that monochloramine was not able to immediately and irreversibly oxidize sulfhydryl groups. Such oxidation would have resulted in the rapid inactivation of the bacteria. They hypothesized that since monochloramine required higher concentrations and longer contact times to destroy bacteria completely and could not readily and irreversibly oxidize the sulfhydryl groups of the glucose oxidation enzymes, its ability to inactivate microorganisms should be attributed to changes in enzymes that may not be involved in the inactivation of the organism by hypochlorous acid. Thus, while the sulfhydryl group may be the most vulnerable to a strong oxidant like hypochlorous acid, changes in other groups produced by the weaker oxidant, monochloramine, may lead also to microbial inactivation. More recent information indicates that the destructive effects of chloramine might be associated with the effects of chloramine on nucleic acids or DNA of cells (Fetner, 1962; Shih and Lederberg, 1976a,b).

Nusbaum (1952) suggested that the disinfective activity of dichloramine occurs by a mechanism similar to monochloramine, but there do not appear to be any data to support this contention.

Considering the mechanism of destruction or inactivation of microorganisms by chlorine and associated compounds, it is interesting to note that Fair et al. (1948) speculated that there might be three or four "targets" or points of attack and that perhaps all must be affected before there is death. This "multiple hit" concept supported the observation that monochloramine must alter groups other than the sulfhydryl group to be effective in the destruction of microorganisms.

Thus, the action of chlorine on microbes such as bacteria and amebic cysts may involve some or all of the steps in the following sequence: penetration of the disinfectant through the cell wall followed by attack on the cell membrane (the site of cellular respiration in bacteria) and disruption of permeability of the cell membrane, which leads to a loss of cell constituents, thereby disrupting metabolic functions within the cell including those involving nucleic acids. Changes in viability may result from this process. Experimental studies on virus (Olivieri *et al*., 1975) demonstrated that chlorine caused initial damage on the viral nucleic acid while leaving the capsid protein unaffected until after the virus was inactivated.

Summary

Chlorine is the most widely used water supply disinfectant in the United States. Depending upon the predominant species of chlorine, hypochlorous acid, and/or hypochlorite ion, disinfection with chlorine can achieve greater than 99.9% destruction of bacteria. For example, a chlorine residual of 0.2 to 1.0 mg/l and a contact time of 15 to 30 minutes will inactivate 99.9% of *E. coli* (Walton, 1969). According to Walton, a properly designed, constructed, and operated water treatment plant, consisting of chemical coagulation, sedimentation, filtration, and disinfection, can remove or destroy more than 99.999% of the coliform bacteria that are present. Although most investigations on the removal or destruction of bacteria have used *E. coli*, there is evidence that the bacterial pathogens, e.g., *Salmonella typhi*, respond somewhat similarly to *E. coli*.

Laboratory studies have demonstrated that that there is limited virus inactivation after the added chlorine has reacted with any ammonia that is in the water. Most inactivation probably occurs in the first few seconds before the chlorine has completed its reaction with ammonia (Olivieri *et al.*, 1971)

Table 3 displays c.t values for *E. coli* and poliovirus inactivation for the various species of free and combined chlorine

Research Recommendations

Recent reports of enhanced chlorine resistance of certain viral and bacterial strains should be investigated and the mechanism of increased resistance elucidated, if the reports are corroborated.

Other recommendations, applicable to other agents as well as to chlorine, are included after the evaluations of the other methods of disinfection.

TABLE 3

Dosages of Various Chlorine Species Required for 99% Inactivation of *Escherichia coli* and Poliovirus 1

Test Microorganism	Disinfecting Agent	Concentration, mg/l	Contact Time, min	c.t[a]	pH	Temperature °C	References
E. coli	Hypochlorous acid (HOCl)	0.1	0.4	0.04	6.0	5	Scarpino *et al.*, 1974
	Hypochlorite ion (OCl^-)	1.0	0.92	0.92	10.0	5	Scarpino *et al.*, 1974
	Monochloramine (NH_2Cl)	1.0	175.0	175.0	9.0	5	Siders *et al.*, 1973 Dorn, 1974
		1.0	64	64.0	9.0	15	Siders *et al.*, 1973 Dorn, 1974
		1.2	33.5	40.2	9.0	25	Siders *et al.*, 1973 Dorn, 1974
	Dichloramine ($NHCl_2$)	1.0	5.5	5.5	4.5	15	Esposito, 1974 Esposito *et al.*, 1974
Poliovirus 1	Hypochlorous acid (HOCl)	1.0	1.0	1.0	6.0	0	Weidenkopf, 1958
		0.5	2.1	1.05	6.0	5	Engelbrecht *et al.*, 1978
		1.0	2.1	2.1	6.0	5	Scarpino *et al.*, 1974
		1.0	1.0	1.0	6.0	15	Brigano *et al.*, 1978
	Hypochlorite ion (OCl^-)	0.5	21	10.5	10.0	5	Engelbrecht *et al.*, 1978
		1.0	3.5	3.5	10.0	15	Brigano *et al.*, 1978
	Monochloramine (NH_2Cl)	10	90	900	9.0	15	Siders *et al.*, 1973 Dorn, 1974
		10	32	320	9.0	25	Siders *et al.*, 1973 Dorn, 1974
	Dichloramine ($NHCl_2$)	100	140	14,000	4.5	5	Esposito, 1974 Esposito *et al.*, 1974
		100	50	5,000	4.5	15	Esposito, 1974 Esposito *et al.*, 1974

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[a] Concentration of compound times contact time (mg/l)(min).

OZONE

Chemistry of Ozone in Water

Ozone has the molecular formula O_3, a molecular weight of 48 g/mol, and a density, as a gas, of 2.154 g/l at 0°C and 1 atm. It is approximately 13 times more soluble in water than is oxygen. At saturation in water at 20°C, a 2% weight mixture of ozone and oxygen contains about 11 mg of ozone and 40 mg of oxygen per liter.

Ozone has a half-life in pure distilled water of approximately 40 minutes at pH 7.6, but this decreases to 10 minutes at pH 8.5 (Stumm, 1958) at 14.6°C. Rising temperatures increase the rate of decomposition. Because its half-life is so short, ozone must be generated on the site where it is to be used.

Ozone is a powerful oxidant that reacts rapidly with most organic and many inorganic compounds. It does not convert chloride to chlorine under test conditions (USEPA, 1976), nor does it react extensively with ammonia (NH_3) (Singer and Zilli, 1975). However, bromide and iodide are oxidized to bromine and iodine. Singer and Zilli (1975) reported that oxidation of ammonia was pH-dependent. At pH 7.0, 9% of a 29 mg/l ammonia nitrogen (NH_3-N) solution was oxidized in 30 minutes, but at pH 9.0 70% of a 24.4 mg/l solution was oxidized in the same time. During disinfection, only minor amounts of ammonia are oxidized when ozone is used. Ozone's limited reaction with ammonia is desirable, but its fast reaction rate with most organic and many inorganic compounds further shortens its persistence in water.

Production and Application of Ozone

Ozone is produced on site from a stream of clean dry air or oxygen by passing an electrical discharge between electrodes that are separated by a dielectric. Approximately twice the percent of ozone by weight is obtained if oxygen, rather than air, is used as the feed stream. Power requirements are about 13 to 22 kWh/kg of ozone that is generated from air and approximately half that when oxygen is used (Rosen, 1972). Compressors and dryers may increase these requirements by 20% to 50%. Other factors affecting efficiency are the rate of gas flow, applied voltage, and the temperature of the gas. The heat that is produced during the process must be removed by cooling with either air or water.

The ozone gas stream must be fed into the water to effect the transfer of ozone. The usual methods are to inject the ozone gas stream through an orifice at the bottom of a co- or countercurrent contact chamber or to aspirate the gas into a contact chamber where it is mixed with the water mechanically. Successful design and operation of the contactor system is necessary to minimize costs of the operation.

Commercial equipment is available in a wide range of capacities--from a few grams of ozone per day to over 40 kg/day. Larger capacities are obtained by adding additional units. Successful delivery of ozone to the water to be treated requires a dependable power supply and reasonably maintenance-free ozonization equipment.

Ozone has been used in a great number of water treatment plants throughout the world. However, in small institutions and private residences, its use appears limited, because it requires dependable power supplies and, usually, a second disinfectant to furnish a disinfecting residual in the system. The maintenance and repairs that are required for the specialized ozone generation equipment provide further barriers against the use of ozone by small institutions.

Analytical Methods

The disinfection process is usually controlled in one of two ways: by the dosage of a specified amount of ozone or by the maintenance of a specified minimum residual for a given time. Residual measurements in both the gas stream and water are sometimes required. Standard Methods (1976) contains descriptions of the measurement of ozone in water by the iodometric, orthotolidine-manganese sulfate, and orthotolidine-arsenite methods. Of these methods the iodometric method, which is subject to the fewest interferences, is the method of choice. Determinations must be made immediately since ozone decomposes rapidly.

In all three methods, the oxidant compounds that result from the reaction of ozone with contaminants in water may react with the test reagents, thereby indicating a higher concentration of ozone than is actually present. This is particularly true in the presence of organic matter, which results in the formation of organic peroxides. In the iodometric method this interference and others are minimized by stripping the ozone from the sample with nitrogen or air and absorbing it from this gas stream in an iodide solution. These and other methods are described in Standard Methods.

Shechter (1973) developed a UV spectrophotometric method to measure the triiodide that is formed by the oxidation of iodide by ozone. She reported a better sensitivity at low ozone concentrations (0.01 to 0.3 mg/l) than achieved with the normal titration method. The effects of interferences on the direct measurement of ozone without sparging the ozone to a separate iodide solution were not indicated. These effects are noted in Standard Methods.

Analytical determination of ozone in water in the presence of other oxidants is poor. Considerable work in this area is needed.

Residual

As discussed above, ozone is unstable in water with a half-life of approximately 40 minutes at pH 7.6 and 14.6°C. Many regard the half-life in water supplies at higher ambient temperatures to be 10 to 20 minutes.

In a recent review of the literature Peleg (1976) concluded that the possible species to be found in an ozone solution were ozone, hydroxyl radicals (OH), hydroperoxyl radicals (HO_2), oxide radicals (O^-), ozonide radicals (O_3^-) and, possibly, free oxygen atoms (O). Hydrogen peroxide (H_2O_2) may also be present by dimerization of the hydroxyl radicals. There have been no studies on the disinfecting activities of these individual species except for those on hydrogen peroxide, which is a poor biocide (when compared to chlorine). Peleg concluded that evidence indicates that the dissociation species are better disinfectants than ozone.

Biocidal Activity

Efficacy Against Bacteria

Wuhrmann and Meyrath (1955) found that 99% of *Escherchia coli* were inactivated in 21 seconds with 0.0125 mg/l residual ozone, in 62 seconds with 0.0023 mg/l residual ozone, and in 100 seconds with 0.0006 mg/l residual ozone at pH 7.0 and 12°C. Spores of a *Bacillus* species were much more resistant, requiring 2 minutes with 0.191 mg/l residual ozone and 5 minutes with 0.049 mg/l residual ozone for 99% inactivation. These data were obtained at pH 7.2 and 22°C. The observed ozone residuals were reported as being constant throughout the test periods.

Katzenelson *et al*. (1974) found that an initial residual ozone concentration of 0.04 mg/l in demand-free water inactivated approximately 3 logs of E. coli in 50 seconds, while a concentration of 1.3 mg/l achieved the same degree of inactivation in 5 seconds. They obtained their inactivation data from experiments in which the loss of initial ozone residuals did not exceed 20%. Their work was conducted at 1°C and pH 7.2. The ozone was determined by the method of Shechter (1973).

Using washed cells of E. coli, Bacillus megaterium, and Bacillus cereus, which were suspended in deionized water with 10^6 cells/ml, and different concentrations of ozone up to 0.71 mg/l, Broadwater et al. (1973) demonstrated that the inactivation of all three test organisms gave an "all-or-none" response. They measured the ozone by stripping it into iodide at the end of the contact period. Consequently, any demand should have used up whatever ozone was needed. They did measure what they presumed to be ozone and not some breakdown product. Thus, they appear to have eliminated the ozone demand problem as much as is possible with present techniques. However, it is possible that their results reflect some effect or artifact not yet understood. With the constant contact time of 5 minutes, no inactivation of vegetative cells of E. coli or B. megaterium occurred until the initial residual concentration of ozone was 0.19 mg/l, when the density of viable cells of both species decreased to "near zero." With the vegetative cells of B. cereus, an initial ozone residual of 0.12 mg/l was required before any inactivation occurred, and then it was nearly complete.

Spores of *B. megaterium* and *B. cereus* were not inactivated until the ozone residual reached approximately 2.29 mg/l, when the spores of both organisms showed an "all-or-none" response. All of the inactivation experiments were performed at 28°C, but the pH was not reported.

Burleson *et al*. (1975) determined that *E. coli*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Shigella flexneri*, *Psuedomonas fluorescens*, and *Vibrio cholerae* were all reduced by 7.5 logs in 15 seconds after exposure to approximately 0.5 mg/l of ozone at 25°C in phosphate-buffered saline. The pH was not reported. In this study, the bacteria were placed in unozonized water (no initial residual), and the ozone was then sparged into the water. After 15 seconds the ozone concentration in solution reached approximately 0.5 mg/l. This technique does not render quantitative data. Ozone was determined by spectrophotometric measurement of iodine that was released from iodide without stripping of the ozone.

The inactivation of *E. coli* with initial counts of about 5 x 10^5/ml in buffered demand-free water at 11°C was measured by Ross *et al*. (1976). For 99% inactivation with 0.1 mg/l of initial ozone residuals, contact times of 16.5 and 21 seconds were required at pH 6 and 10, respectively. Ozone was measured by the diethyl-p-phenylenediamine (DPD) method.

Farooq (1976) and Farooq *et al*. (1977a, b, c) studied the effects of pH and temperature in ozone inactivation studies with Mycobacterium fortuitum and Candida parapsilosis (a yeast). They concluded that if the ozone residual remains constant, the disinfection capability will not be affected by a change in pH. They also demonstrated that for a given dosage a rise in temperature increases the rate of inactivation even though the ozone residual was decreased. (Ozone was less soluble at higher temperature). Vorchinskii (1963) concluded that whereas the bactericidal dose of ozone at 4°C to 6°C was unity, the corresponding dose at 18°C to 21°C was 1.6 and at 36°C to 38°C was 3.6.

The work of Farooq and his colleagues is in agreement with that of Morris (1976), who observed that the disinfection capability of ozone does not change significantly with pH, at least over the normal pH range (6 to 8.5) of water supplies.

Efficacy Against Viruses

Katzenelson et al. (1974) investigated the inactivation of poliovirus 1 at 5°C and pH 7.2. A 99% inactivation of the virus occurred in less than 8 seconds with 0.3 mg/l of initial residual ozone. Their experimental methods were the same as those described above for bacteria. They also observed that inactivation resulted from two distinct stages (rates) of action. The first stage of inactivation, less than 8 seconds long, produced a virus inactivation of from 99% to 99.5%, depending upon the ozone residual. The second stage, which lasted from 1 to 5 minutes, still left some viruses infective. Additional work showed that the slower second-stage inactivation apparently involved the inactivation of viruses that were clumped together. The single virus particles were inactivated during the first stage. After ultrasonic treatment, 99.5% of the virus was inactivated within 8 seconds and more than 99.99% within 3 minutes at an initial ozone residual of 0.1 mg/l.

Coin et al. (1964) investigated the inactivation of poliovirus 1 (Mahoney) in a batch system in which a measured amount of ozone was introduced into a virus suspension in distilled and filtered river water. In distilled water nearly 1 log of the virus was inactivated in 4 minutes at a 4-minute ozone residual of approximately 0.23 mg/l. In river water the inactivation was in excess of 99.99% in 4 minutes when the ozone concentration, also measured at 4 minutes, exceeded 0.3 mg/l. The ozone was measured by the iodide titration without stripping of the ozone. Rather large ozone demands existed in this system. An initial 5 mg/l residual ozone concentration in the distilled water decreased to about 0.6 to 0.8 mg/l in 4 minutes. The same initial residual in river water decreased to between 0.2 to 0.6 mg/l. Temperature and pH were not reported.

Keller et al. (1974) studied ozone inactivation of viruses in a water supply by using both batch tests and a pilot plant with a 38 l/minute (10 gallons/minute) flow rate. Inactivation of poliovirus 2 and coxsackievirus B3 in 5 minutes was greater than 99.9% in the batch tests when the ozone residual was between 0.8 and 1.7 mg/l at the end of the 5-minute contact period. Initial ozone residuals varied from 1.6 to 2.8 mg/l. At the pilot plant, greater than 99.999% inactivation of coxsackievirus B3 could be achieved with an ozone dosage of 1.45 mg/l, which provided an ozone residual of 0.28 mg/l after 1 minute in lake water. Temperature and pH conditions were not reported. Ozone was measured by the iodide method without prior stripping of the ozone.

Burleson _et_ _al_. (1975) obtained inactivations of >99.9% for vesicular stomatitis virus, >99.99% for encephalomyocarditis virus, and 99.99% for GD VII virus in 15 seconds with an ozone residual of approximately 0.5 mg/l of ozone. The experiment was conducted in the same manner as that described above for their studies with bacteria.

Evison (1977) reported data for the inactivation of a number of viruses in buffered water at pH 7.0 and 25°C (Table 4).

The reported ozone concentrations were evidently those measured initially and maintained by the addition of ozone during the experiment. Ozone was measured by a colorimetric version of Palin's DPD technique. The Evison data show that more ozone or a longer time are required for inactivation than do the data of other workers. This may have resulted from the virus purification used. Her viruses were purified by low-speed centrifugation and filtration through an 0.2-μm membrane filter. These clean-up procedures are neither as

TABLE 4

Ozone Required for Inactivation of Viruses in 10 minutes at pH 7.0 and 25°C[a]

	Ozone Required, mg/1	
Virus	99.9% Inactivation	99% Inactivation
Coxsackie B3	0.6	0.095
Polio 3	0.22	0.082
Polio 1	0.095	0.042
Echo 1	0.086	0.044
Coxsackie B5	0.076	0.053
Polio 2	0.052	0.039

[a] Data from Evison, 1977.

complete nor as thorough as those used by other investigators. The unremoved cell debris and organic matter offer protection to the virus. Either higher ozone residuals or longer contact times would be required to inactivate such preparations to the same extent as clean virus. Other data showed that the inactivation of coliphage 185 was relatively unaffected by pH's ranging from 6.0 to 8.0 at ozone concentrations exceeding 0.05 to 0.10 mg/l. Evison (1977) also concluded that the rate of inactivation of the coliphage 185 by ozone was much less affected by temperature than was the inactivation by chlorine.

Farooq (1976) observed a 99% inactivation of 10^5/ml poliovirus 1 in 30 seconds in distilled water at an initial ozone residual concentration between 0.23 and 0.26 mg/l at 24°C and pH 7.0. In this study, ozone was measured by the Shechter (1973) method.

Sproul *et al*. (1978) reported total inactivation in 10 seconds of poliovirus 1 (Sabin), which had initial concentrations of 1.3×10^2 to 2.4×10^3 plaque-forming units (PFU)/ml. The initial ozone residual of 0.012 to 0.085 mg/l decreased by approximately one-third in 40 seconds. The experiments were conducted with the Sharpe dynamic reactor. Ozone was measured by the Shechter (1973) method.

Efficacy Against Parasites

No studies on the efficacy of ozone as an antihelmintic agent appear to have been reported.

Ozone may have application as an antiparasitic agent in the treatment of water supplies but only limited information is available. Newton and Jones (1949) reported that ozone, with 5-minute residuals as low as 0.3 mg/l, inactivated from 98% to >99% of Entamoeba histolytica cysts that were suspended in water. Initial ozone residuals that were required to obtain 5-minute residuals of 0.3 mg/l varied from 0.7 mg/l to 0.9 mg/l. With the ozone concentrations used, the cysticidal action was not affected by temperatures from 10°C to 27°C nor by pH's between 6.5 to 8.0. Ozone was measured by titration of iodine, which was released from iodide directly in the reactors without removal of ozone by sparging.

Mechanism of Action

Investigations of the inactivation of bacteria by ozone have centered on the action of ozone on the cell membrane. Scott and Lesher (1963) concluded from their work with E. coli that the primary attack of ozone occurred on the double bonds of the fatty acids in the cell wall and membrane and that there was a consequent change in the cell permeability. Cell contents then leaked into the water. This was confirmed by Smith (1967). Prat et al. (1968), examining extracts from ozonized E. coli, reported that thymine was more sensitive to ozone attack than were cytosine and uracil.

Riesser et al. (1977) showed that ozone attacked the protein capsid of poliovirus 2 in such a manner that the virus was not taken up into susceptible cells. An electrophoretic study showed complete loss of viral proteins in a poliovirus 2 sample that had showed an inactivation of 7 logs in 20 minutes.

Summary and Conclusions

Inactivation with ozone at specified ozone residuals is relatively insensitive to pH's between 6 and 8.5. Moreover, ozone does not react with ammonia over this same range when short detention times are used. The data on temperature are not sufficiently firm to permit conclusions concerning its effect on disinfection. Ozone must be generated on site, and the process is relatively energy intensive. To make economic comparisons of ozone with other disinfectants, the cost of local power must be ascertained.

Available kinetic data on ozone inactivation are presented in Table 5. The c.t product for 99% inactivation of E. coli appears to be approximately 0.006 at near-neutral pH values and at temperatures of ≤10°C. There was less consistency for the c.t products of poliovirus 1. These values varied from <0.005 to 0.42 at pH 7 and at temperatures from 5°C to 25°C.

TABLE 5

Concentration of Ozone and Contact Time Necessary for 99% Inactivation of *Escherichia coli* and Polio 1 virus

Test Microorganism	Ozone mg/1	Contact Time, min	c.t[a] (mg/1)(min)	pH	Temperature, °C	References
E. coli	0.07	0.083	0.006	7.2	1	Katzenelson *et al.*, 1974
	0.065	0.33	0.022	7.2	1	Katzenelson *et al.*, 1974
	0.04	0.50	0.02	7.2	1	Katzenelson *et al.*, 1974
	0.01	0.275	0.027	6.0	11	Ross *et al.*, 1976
	0.01	0.35	0.035	6.0	11	Ross *et al.*, 1976
	0.0006	1.7	0.001	7.0	12	Wuhrmann and Meyrath, 1955
	0.0023	1.03	0.002	7.0	12	Wuhrmann and Meyrath, 1955
	0.0125	0.33	0.004	7.0	12	Wuhrmann and Meyrath, 1955
Polio 1	<0.3	0.13	<0.04	7.2	5	Katzenelson *et al.*, 1974
	0.245	0.50	0.12	7.0	24	Farooq, 1976
	0.042	10	0.42	7.0	25	Evison, 1977
	<0.03	0.16	<0.005	7.0	20	Sproul *et al.*, 1978

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[a] Concentration of ozone times contact time.

The c.t products vary over a broad range. These variations illustrate the difficulty of doing quantitative experimentation with ozone and microorganisms in water. Among other reasons these difficulties are caused by undetected ozone demand in the water, poor analytical techniques for residual ozone, and nonuniformity of microorganisms from one laboratory to another. As an example of the latter, different strains of poliovirus with different inactivation rates are used, but the inactivation data are frequently not reported as strain-specific. Furthermore, viruses frequently exist in an undetected clumped state rather than in the presumed single discrete particle state. Higher c.t products are required for the clumped viruses than for the unclumped ones.

Because of ozone's relatively short half-life in water, another disinfectant must be added to maintain a disinfection capability in the distribution system. The most effective disinfectant, its optimum concentration, and method of addition must be determined. The disinfection process with ozone will probably be controlled by specifying the ozone residual at the beginning and end of a given contact time.

Research Recommendations

Future research studies with ozone should be conducted to:

-- provide data on the inactivation of enteric pathogens;
-- provide more data on the inactivation of protozoan cysts, especially those of *Giardia lamblia*;
-- provide analytical methods that are specific for ozone;
-- provide additional definitive data on bacteria and viruses, and eliminate discrepancies; and
-- provide operating data from full-scale drinking water plants to demonstrate reliability of operation, operating costs, ozone dosages, residuals and contact times, and disinfection results.

CHLORINE DIOXIDE

Chlorine dioxide (ClO_2) was first prepared in the early nineteenth century by Sir Humphrey Davey (1811). By combining potassium chlorate ($KClO_3$) and hydrochloric acid (HCl), he produced a greenish-yellow gas, which he named "euchlorine". Later, this gas was found to be a mixture of chlorine dioxide and chlorine. The bleaching action of chlorine dioxide on wood pulp was recognized by Watt and Burgess (1854) and by Schmidt (1921).

Large quantities of chlorine dioxide are produced each day in the United States. Although its primary application has been the bleaching of wood pulp, it is also used extensively for bleaching and dye stripping in the textile industry and for bleaching flour, fats, oils, and waxes (Gall, 1978).

In the United States, chlorine dioxide was first used in 1944 at the water treatment plant in Niagara Falls, New York to control phenolic tastes and odors arising from the presence of industrial wastes, algae, and decaying vegetation (Synan et al., 1945). Granstrom and Lee (1958) surveyed water treatment plants believed to be using chlorine dioxide. The majority of respondents (956 plants) were using it for taste and odor control. Other uses reported were algal control (7 plants), iron and manganese removal (3 plants), and disinfection (15 plants).

Sussman (1978) compiled a partial listing of plants using chlorine dioxide. He reported that the compound is used primarily to control taste and odor in the United States. In England, Italy, and Switzerland, it is used for disinfection of water supplies.

The Chemistry of Chlorine Dioxide in Water

Chlorine dioxide reacts with a wide variety of organic and inorganic chemicals under conditions that are usually found in water treatment systems (Stevens *et al.*, 1978). However, two important reactions do not occur. Chlorine dioxide *per se* does not react to cause the formation of trihalomethanes (THM's) (Miltner, 1976). However, THM's will be formed if the chlorine dioxide is contaminated with chlorine. Such a situation may occur when chlorine is used in the preparation of chlorine dioxide.

Chlorine dioxide does not react with ammonia, but will react with other amines (Rosenblatt, 1978). The amine structure determines reactivity. Tertiary amines are more reactive with chlorine than secondary amines, which, in turn, are more reactive than primary amines.

Production and Application

Chlorine dioxide condenses to form an unstable liquid. Both the gas and liquid are sensitive to temperature, pressure, and light. At concentrations above 10% in air, chlorine dioxide may be explosive, and at 4% in air, it can be detonated by sparks (Gall, 1978; Sussman, 1978). As a result, the preparation and distribution of chlorine dioxide in bulk have not been deemed practical. It has been generated and used on site.

Sodium chlorate ($NaClO_3$) or sodium chlorite ($NaClO_2$) may be used to generate chlorine dioxide. The method of production will depend upon the amount of chorine dioxide that is required. The reduction of sodium chlorate is the more efficient process and is generally used when large volumes and high concentrations of chlorine dioxide are needed. Commercial processes that are used in North America for large-scale production of chlorine dioxide are based on the three reactions listed below. To reduce the sodium chlorate, each process uses a different agent: sulfur dioxide (SO_2), methanol (CH_3OH), and the chloride ion (Cl^-).

$$2NaClO_3 + H_2SO_4 + SO_2 \rightarrow 2ClO_2 + 2NaHSO_4 \quad (6)$$

$$2NaClO_3 + CH_3OH + H_2SO_4 \rightarrow 2ClO_2 + HCHO + Na_2SO_4 + 2H_2O \quad (7)$$

$$NaClO_3 + NaCl + H_2SO_4 \rightarrow ClO_2 + 1/2Cl_2 + Na_2SO_4 + H_2O \quad (8)$$

All of these processes are used in the pulp and paper industry. They can also be used to prepare chlorine dioxide for the large waterworks that might require several metric tons per day. Small quantities of chlorine are formed during the side reactions and intermediate reactions in these processes. A more detailed review of the chemistry that is involved in the production of chlorine dixide from chlorate is given by Gall (1978) and Gordon _et al_. (1972).

Chlorine dioxide can be prepared from chlorine and sodium chlorite through the following reactions:

$$Cl_2 + H_2O \rightarrow HOCl + HCl \qquad (9)$$

$$HOCl + HCl + 2NaClO_2 \rightarrow 2ClO_2 + 2NaCl + H_2O \qquad (10)$$

The theoretical weight ratio of sodium chlorite to chlorine is 1:0.39 (Dowling, 1974). With available sodium chlorite (80%), the weight ratio is 1:0.30. In practice, Gall (1978) recommended a chlorite to chlorine ratio of 1:1. The excess chlorine lowers the pH, thereby increasing the reaction rate and optimizing the yield of chlorine dioxide. Dowling (1974) reported that the maximum theoretical yield of chlorine dioxide was produced when the ratio was normally maintained at a minimum of 1:0.5.

Alternatively, chlorine dioxide may be prepared from sodium hypochlorite (NaOCl) and sodium chlorite. The sodium hypochlorite is acidified to yield hypochlorous acid (HOCl), and the chlorine dioxide is generated according to Reaction 10. Each of the methods produces a solution containing both chlorine and chlorine dioxide.

Chlorine dioxide may also be prepared by the addition of a strong acid, such as sulfuric acid (H_2SO_4) or hydrochloric acid, to sodium chlorite as shown in the following reactions:

$$10NaClO_2 + 5H_2SO_4 \rightarrow 8ClO_2 + 5Na_2SO_4 + 2HCl + 4H_2O \qquad (11)$$

$$5NaClO_2 + 4\ HCl \rightarrow 4\ ClO_2 + 5\ NaCl + 2\ H_2O \qquad (12)$$

Although some investigators have claimed that this method produces chlorine-free chlorine dioxide, Reuss (1964) and

Schilling (1956) reported that chlorine is also formed. Dowling (1974) indicated that chlorine was formed even when sulfuric acid was used.

Analytical Methods

Chlorine dioxide is one of the few stable nonmetallic inorganic free radicals (Rosenblatt 1978) .It does not contain available chlorine in the form of hypochlorous acid or hypochlorite ion (HOCl−). However, concentrations of chlorine dioxide are often reported in terms of available chlorine. The chlorine atom in chlorine dioxide has a valance of +4. A reduction to chloride results in a gain of five electrons. In terms of available chlorine, chlorine dioxide has 263% or more than 2.5 times the oxidizing capacity of chlorine.

$$\frac{5e^{-} X\ 35.45}{67.45} \ X\ 100 = 263\% \qquad (13)$$

The weight ratio of chlorine dioxide to available chlorine is 67.45 to 35.45 or 1.9. However, in water treatment practices this increased oxidizing capacity is rarely realized. The reduction of chlorine dioxide depends heavily on pH and the nature of the reducing agent. At neutral or alkaline pH, chlorine dioxide is reduced to chlorite, a net gain of one electron. Thus, only one-fifth or 20% of its oxidizing capacity is utilized. At low pH, the chlorite (ClO_2^-) is reduced to chloride (Cl−) releasing the remaining four available electrons.

Chlorine dioxide may be determined iodometrically (Standard Methods, 1976), amperometrically (Haller and Listek, 1948; Standard Methods, 1976), spectrophotometrically (Gordon et al., 1972), and colorimetrically (Aston, 1950; Hodgen and Ingols, 1954; Masschelein, 1966; Palin, 1957, 1960, 1967, 1970, 1974, 1975; Post and Moore, 1959; Standard Methods, 1976).

Several studies contain comparisons of various analytical methods and procedures for the measurement of chlorine dioxide. Adams et al. (1966) compared the H-acid, tyrosine, amperometric, and diethyl-p-phenylenediamine (DPD) procedures for free chlorine, chlorine dioxide, and chlorite. They reported DPD to be the most reliable as did Dowling (1974), Miltner (1976), and the U.S. Public Health Service (Miltner, 1976). Myhrstad and Samdal (1969) noted that the DPD method (Palin, 1957, 1960) yielded consistently higher residual measurements for chlorine dioxide than those that are produced with other analytical methods. After analysis with acid chrome violet K (Masschelein, 1966), chlorine dioxide was not observed in the water of the distribution system; however, chlorite was found. The residuals that were previously interpreted as chlorine dioxide were apparently due to chlorite.

Recently, more sophisticated procedures were suggested for the determination of chlorine dioxide. Moffa et al. (1975) reported the use of electron spin resonance, and Issacsson and Wettermark (1976) described a chemiluminescent method for active chlorine compounds. Stevenson et al. (1978) reviewed electrochemical methods and presented preliminary results for a membrane amperometric probe. Under development is a sensor that shows a linear response region from about 0.5 to 10 mg/l. The response to hypochlorous acid and chloramines was low, and the sensor does not measure chlorite or other ionized species.

No one procedure appears to possess the necessary sensitivity, selectivity, and simplicity to permit reliable determinations in the treatment of water. Each of the titration methods are prone to error because of volatilization. They are time-consuming and particularly complex when differentiation of chlorine and oxychloro species are necessary. The colorimetric procedures require strict control of pH, temperature, and reaction times and will be affected by turbidity. In addition, the selectivity of the indicators for chlorine dioxide is questionable. The direct spectrophotometric determination of chlorine dioxide at 360 nm is selective and rapid but is not sufficiently sensitive for use in water. Limited experience with the more recent procedures (chemiluminescence and membrane amperometric probe) does not permit an evaluation.

In practice, the principal distinction that must be made is that between the active biocidal species (hypochlorous acid, the hypochloride ion, and chlorine dioxide), the moderately biocidal species--(monochloramine [NH_2Cl], dichloramine [$NHCl_2$], and nitrogen trichloride [NCl_3]), and the relatively nonbiocidal species (chlorite and chlorate [ClO_3^-] ions). This is imperative when the primary purpose for the addition of chlorine dioxide is the inactivation of microorganisms. Furthermore, when the formation of THM's is to be considered, the distinction between free chlorine and chlorine dioxide becomes important.

Biocidal Activity

Efficacy Against Bacteria

Experimental data on the efficacy of chlorine dioxide as a disinfectant became available in the early 1940's. McCarthy (1944, 1945) reported that chlorine dioxide was an effective bactericide in water with a low organic content. When the levels of organic material in the water were high, chlorine dioxide was less effective.

Ridenour and Ingols (1947) reported that chlorine dioxide was at least as effective as chlorine against *Escherichia coli* after 30 minutes at similar residual concentrations. Both chlorine and chlorine dioxide residues were determined by the orthotolidine-arsenite (OTA) method. The bactericidal activity of chlorine dioxide was not affected by pH values from 6.0 to 10.0. Ridenour and Armbruster (1949) extended their observation to other enteric bacteria. The common waterborne pathogens were similarly inactivated with chlorine dioxide. They also reported that the efficiency of chlorine dioxide decreased as the temperature decreased from 20°C to 5°C.

Ridenour _et al_. (1949) found chlorine dioxide to be more effective than chlorine (based on OTA residuals) against bacterial endospores. They indicated that less weight of chlorine dioxide than chlorine is required to inactivate the spores of _Bacillus mesentericus_ in either demand-free water or in waters containing ammonia. In the waters containing ammonia, chlorine had to be applied beyond breakpoint before efficient sporicidal activity was observed.

The work of Ridenour and colleagues is not discussed in depth because the small amounts of free chlorine that are produced during the generation of chlorine dioxide are not distinguished from the chlorine dioxide by the OTA method that they used for both stock solutions and residual determination. In their 1947 paper (Ridenour and Ingols, 1947), the survival measurements were ± and not quantitative. In their 1949 paper (Ridenour and Armbruster, 1949), the survival measurement was quantitative, but only one contact time was used, 5.0 minutes. Since chlorine dioxide is a rapdily acting disinfectant, c.t products may be misleading using this contact time.

Russian investigators (Bedulevich _et al_., 1953; Trakhtman, 1946) found chlorine dioxide to be a more effective or at least as effective a bactericide as chlorine. Additional data were reported for _Bacillus anthracis_. They observed that the efficiency of chlorine dioxide decreased as the pH of the system containing the B. anthracis increased.

Early studies on disinfectant activity are difficult to interpret because the methods of preparing chlorine dioxide invariably included the addition or production of chlorine. Analytical procedures were not sufficiently advanced to differentiate between chlorine dioxide and other oxychloro species. Thus, the quantitative analyses of stock solutions and reports of dose and residual chlorine dioxide may be in error. This suggests that the initial and residual concentrations of chlorine dioxide were probably lower than reported values and that the comparative bactericidal efficiency would suffer accordingly. In addition, the older investigations did not take into account the volatility of chlorine dioxide. Depending upon concentration and length of exposure, losses of 7% to 30% can occur within an hour.

Many of the difficulties that were encountered during the early studies were overcome in a series of studies reported by Benarde and coworkers during the mid-1960's. Their work on disinfection was based heavily on the improved methods of preparing and analyzing chlorine dioxide, which were reported by Granstrom and Lee (1958). They prepared chlorine dioxide by oxidizing sodium chlorite with persulfate ($S_2O_8{}^{2-}$) under acid conditions. The resulting chlorine dioxide was swept to a collection vessel by high purity nitrogen gas. Chlorine dioxide was measured spectrophotometrically at 357 nm.

Bernarde et al. (1965) compared the bactericidal effectiveness of chlorine with that of chlorine dioxide at pH 6.5 and 8.5 in a demand-free buffered system. At pH 6.5, both chlorine and chlorine dioxide inactivated a freshly isolated strain of *E. coli* in less than 60 seconds. Chlorine was slightly more effective at the lower dosages at the lower pH. At pH 8.5, chlorine dioxide was dramatically more effective than chlorine. Greater than 99% inactivation of *E. coli* was observed in 15 seconds with 0.25 mg/l chlorine dioxide, while under the same conditions, chlorine required almost 5 minutes for similar inactivation. Chlorine dioxide was significantly more efficient than chlorine in the presence of high levels of organic and nitrogenous material. Bernarde et al. (1967a) also reported that temperature affects the rate of inactivation of bacteria with chlorine dioxide. A decrease in disinfectant activity was observed as temperature decreased from 30°C to 5°C. For 99% inactivation of *E. coli* with 0.25 mg/l of chlorine dioxide; 190, 74, 41, and 16 seconds were required at 5°C and 10°C, 20°C and 30°C, respectively. More recent work by Cronier (1977) in a clean system also demonstrated the excellent bactericidal activity of chlorine dioxide. Results of both studies are shown in Table 6.

Efficacy Against Viruses

In the mid-1940's, there were also investigations on the virucidal activity of chlorine dioxide. Ridenour and Ingols

TABLE 6

Concentrations of Chlorine Dioxide and Contact Times Necessary for 99% Inactivation of *Escherichia coli*

Test Microorganism	Chlorine Dioxide, mg/l	Contact Time, min	c.t[b]	pH	Temperature, °C
E. coli (freshly isolated from feces)[a]	0.25	1.8	0.45	6.5	5
	0.50	0.83	0.41	6.5	5
	0.75	0.50	0.38	6.5	5
	0.25	1.2	0.30	6.5	10
	0.50	0.47	0.24	6.5	10
	0.75	0.3	0.23	6.5	10
	0.25	0.68	0.17	6.5	20
	0.50	0.35	0.18	6.5	20
	0.75	0.25	0.19	6.5	20
	0.25	0.27	0.07	6.5	32
	0.50	0.22	0.11	6.5	32
	0.75	0.15	0.11	6.5	32
E. coli (ATCC 11229)[c]	0.30	1.8	0.54	7.0	5
	0.50	0.98	0.49	7.0	5
	0.80	0.58	0.41	7.0	5
	0.30	1.3	0.39	7.0	15
	0.50	0.75	0.38	7.0	15
	0.80	0.47	0.38	7.0	15
	0.30	0.98	0.29	7.0	25
	0.50	0.55	0.28	7.0	25
	0.80	0.35	0.28	7.0	25

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[a] Data from Bernarde *et al.*, 1967a.

[b] Concentration of chlorine dioxide times contact time.

[c] Data from Cronier, 1977.

(1946) reported that chlorine dioxide was as effective as chlorine against a mouse-adapted strain of poliovirus (Lansing). Again, their comparison was based upon OTA-determined residual levels of each disinfectant. Hettche and Ehlbeck (1953) found chlorine dioxide to be more effective against poliovirus than either chlorine or ozone. In addition to the difficulties that are associated with the preparation and measurement of chlorine dioxide and chlorine, the early virus studies were also saddled with difficult and time-consuming virus test systems.

The first definitive work on the virucidal activity of chlorine dioxide was done in Göteborg, Sweden. Warriner (1967) showed that the rate of inactivation of poliovirus 3 increased with increasing pH at pH values of 5.6 to 8.5 (see Table 7). Similar to the action on bacteria, the viral inactivation occurred rapidly. When chlorine and chlorine dioxide were combined, the inactivation was synergistic. The inactivation with the two chlorine species together was more efficient at lower pH, but the presence of chlorine dioxide enhanced inactivation by chlorine at the higher pH.

Cronier (1977) compared the inactivation of _E. coli_ (ATCC 11229), poliovirus 1, and coxsackievirus A9. All of the microorganisms that were tested were sensitive to low concentrations (<1 mg/l) of chlorine dioxide. Poliovirus 1 and coxsackievirus A9 were more resistant than _E. coli_. Similar to its bactericidal activity, chlorine dioxide was more effective as a virucide at higher pH. Cronier also

TABLE 7

Concentrations of Chlorine Dioxide and Contact Times Necessary for 99% Inactivation of Viruses

Test Microorganism	Chlorine Dioxide, mg/l	Contact Time, min	c.t[b]	pH	Temperature, °C
Poliovirus 3[a]	0.5	5.0	2.5	5.6	20
	0.5	5.0	2.5	7.2	20
	0.5	0.25	0.125	8.5	20
	1.6	5.0	8.00	5.6	20
	1.6	1.0	1.60	7.1	20
	1.6	0.25	0.40	8.0	20
Poliovirus 1[c]	0.3	16.6	5.0	7.0	5
	0.5	12.0	6.0	7.0	5
	0.8	6.8	5.4	7.0	5
	0.3	4.2	1.3	7.0	15
	0.5	2.5	1.25	7.0	15
	0.8	1.7	1.4	7.0	15
	0.3	3.6	1.08	7.0	25
	0.5	2.0	1.0	7.0	25
	0.8	1.5	1.2	7.0	25
Coxcackievirus A9	0.3	1.2	0.4	7.0	15
	0.5	0.05	0.34	7.0	15
	0.8	0.25	0.20	7.0	15

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[a] Data from Warriner, 1967.

[b] Concentration of chlorine dioxide times contact time.

[c] Data from Cronier, 1977.

reported that on a weight basis, it was similar to hypochlorous acid and better than hypochlorite ion, monochloramine, and dichloramine.

The effect of bentonite ($AlO_3 \bullet 4SiO_2 \bullet H_2O$) (added to the test as a model for turbidity) on disinfection with chlorine dioxide was studied by Scarpino *et al*. (1977). At turbidity levels below 2.29 nephelometric turbidity units (NTU) at 25°C, no protection was afforded to poliovirus 1 by the bentonite. However, at 3.22 and 14.1 NTU's, poliovirus inactivation was noticeably decreased. The bentonite at these levels appeared to offer protection to the virus.

Efficacy Against Parasites

The data that are available on the efficacy of chlorine dioxide on helminths or protozoan cysts do not appear to be suitable for comparison with the action of other disinfectants.

Comparative Biocidal Activity

The data presented in Tables 6 and 7 were collected for demand-free systems within the last 15 years when relatively reliable chemical methods and/or quantitative biocidal assay procedures were used. Table 6 shows the concentration of chlorine dioxide necessary for 99% inactivation of *E. coli* strains. Even at 5°C and chlorine dioxide concentrations of 0.25 to 0.30 mg/l, less than 2 minutes was required for 99% inactivation. Increases in chlorine dioxide concentration and/or temperature markedly reduced the contact time that was necessary. Table 7 presents similar data for viruses. Increased chlorine dioxide concentration, temperature, and pH decreased the contact time that was required to produce 99% inactivation of the viruses. The amount of inactivation depended on the virus that was tested.

Mechanism of Action

There is little information concerning the mode of action by which chlorine dioxide inactivates bacteria and viruses. Ingols and Ridenour (1948) suggested that the bactericidal effectiveness of chlorine dioxide is due to its adsorption on the cell wall with subsequent penetration into the cell where it reacts with enzymes containing sulfhydryl groups. Benarde et al. (1967b) demonstrated that chlorine dioxide abruptly inhibited protein synthesis. The incorporation of ^{14}C-labeled amino acids into protein by whole cells stopped within a few seconds after the addition of chlorine dioxide. Subsequently, Olivieri (1968) reported a dose-response in the inhibition of protein synthesis in bacteria that had been treated with chlorine dioxide. The site of action was localized in the soluble portion (enzymes) of the cell extracts of treated cells without affecting the integrity of the ribosomes' function in protein synthesis.

Conclusion

Chlorine dioxide is an effective bactericide and virucide under the pH, temperature, and turbidity that are expected in the treatment of potable water. It should be noted that the Environmental Proteciton Agency has set an interim Maximum Contaminant Level of 1 mg/l on chlorine dioxide (USEPA, 1978) because of the unresolved questions on its health effects (Symons et al., 1977).

Research Recommendations

A simple, selective, and sensitive test for chlorine dioxide should be developed to monitor residual concentration.

Chlorine dioxide is currently used at several plants. A review of plant records and field studies on the stability and effectiveness of chlorine dioxide in the distribution system should be undertaken.

Studies should be directed toward evaluating the inactivation of protozoan cysts.

More information should be obtained on the mode of action by which chlorine dioxide inactivates bacteria, viruses, and cysts.

IODINE

The use of iodine as a biocide has had a long history, primarily as an antiseptic for skin wounds and mucous surfaces of the body and, to a lesser degree, as a powerful sanitizing agent in hospitals and laboratories (Gershenfeld, 1977). The use of iodine as a disinfectant of drinking and swimming-pool water has not been extensive mainly because of the costs and problems that are involved in applying the dosage.

Aside from the emergency iodination of small volumes for field and emergency drinking water and limited experience with swimming-pool disinfection (Black _et_ _al_., 1959), the only substantial experience with iodine disinfection of piped water system is that of Black _et_ _al_. (1965, 1968). Two water systems serving three prisons in the State of Florida were disinfected satisfactorily with 1 mg/l of iodine. A persistent residual was maintained throughout the distribution system despite a finished water pH of 8 to 9.5. No adverse effects on health were observed among those consuming the water.

Chemistry of Iodine in Water

Iodine is the only common halogen that is a solid at room temperature, and it possesses the highest atomic weight (126.91). Of the four common halogens it is the least soluble in water, has the lowest standard oxidation potential for reduction to halide, and reacts least readily with organic compounds.

The Hydrolysis of Iodine

$$I_2 + H_2O \rightleftharpoons HOI + H^+ + I^- \qquad (14)$$

Diatomic iodine (I_2) reacts with water to form hypoiodous acid (HOI) and iodide ion (I^-). The effect of pH on this reaction is shown in Table 8.

The distribution of chemical species of iodine given in Table 8 was taken from the calculations that were made by Chang (1958) from the equilibrium expression:

$$\frac{[HOI][H]^+[I]^-}{[I_2]} = K_h \qquad (15)$$

The value of K_h, the hydrolysis constant, is given by Wyss and Strandskov (1945) as 3×10^{-12} at 25°C. With iodine residuals at 0.5 mg/l, which are expected in water systems, and a pH of 5, approximately 99% of the total iodine residual is present as iodine and only 1% as hypoiodous acid. At pH 7, the two forms are present in almost equal concentrations. At pH 8, only 12% is present as elemental iodine and 88% as hypoiodous acid, which can be converted to hypoiodite ion (OI^-).

$$HOI \rightleftharpoons H^+ + OI^- \qquad (16)$$

$$\frac{[H^+][OI^-]}{[HOI]} = K_a = 4.5 \times 10^{-12} \qquad (17)$$

TABLE 8

Hydrolysis of Iodine at 25°C Showing the Percentage of Iodine Species at Different pH's[a]

	Percentage of Iodine Species by Concentration of Iodine								
	0.5 mg/1			5.0 mg/1			50.0 mg/1		
pH	I_2	HOI	OI^-	I_2	HOI	OI^-	I_2	HOI	OI^-
5	99	1	0	100	0	0	100	0	0
6	90	10	.0	99	1	0	100	0	0
7	52	48	0	88	12	0	97	3	0
8	12	88	.005	52	48	0	86	14	0

---

[a] Data from Chang, 1958.

$$[H^+] = K_a \frac{[HOI]}{[OI^-]} \qquad (18)$$

$$\frac{[\ HOI^-]}{[OI^-]} = \frac{H^+}{Ka} \qquad (19)$$

The dissociation constant, K_a, of hypoidous acid (at 20°C) is 4.5×10^{-13}. Consequently, the dissociation of hypoiodous acid, which occurs at high pH's, is not important for practical purposes. However, as confirmed by the field studies in Florida, hypoiodous acid can form iodate ion by autooxidation at pH values above 9.

$$3HOI + 2OH^- \rightleftharpoons HOI_3 + 2H_2O + 2I^- \qquad (20)$$

The iodate ion possesses no disinfecting ability (Marks and Strandskov 1950).

Production and Application

Iodine may be added to a municipal water supply by several procedures. One method is to employ nonhazardous solvents and solubilizing agents such as ethyl alcohol (C_2H_5OH) and potassium iodide (KI) to overcome the low concentration of aqueous iodine stock for solution feeders. Another method produces the required concentration of iodine by passing water through a bed of crystalline iodine (saturator). This has been used in many small, semipublic and private home water systems. Since the maximum concentration is limited by solubility to 200-300 mg/l at the ambient temperatures that are expected for drinking water, some physical complications would accompany the introduction of this method into the large waterworks system in view of the large saturation beds required. The iodinated anion exchange resin bed and the vaporization technique are not sufficently developed to be considered for use in public water supplies.

In certain circumstances, potassium iodide might be combined with an oxidation reaction to release iodine. The chemistry is simple, and the persistence of the iodine that is generated may be much better than chlorine. This method has been used in swimming pools and for dechlorination purposes where a chlorine residual may be exchanged for an iodine residual, or an iodine residual may be provided where ozone is the primary disinfectant.

Analytical Methods

Both amperometric titration and leuco crystal violet (LCV) colorimetric methods give acceptable results when used to measure free iodine in drinking water. Waters containing oxidized forms of manganese interfere with the LCV method. Also, when iodide ion exceeds 50 mg/l and chloride ion exceeds 200 mg/l, the amperometric method is preferred. Under unusual situations, where mixtures of chlorine, bromine, and ozone occur along with iodine the problem of separation is difficult (Standard Methods, 1976).

Biocidal Activity

Table 9 shows the relative resistance of bacteria, viruses, and cysts to inactivation by iodine.

Efficacy Against Bacteria

Chang and Morris (1953), summarizing the development of a universal water disinfectant tablet for the military, concluded that iodine concentrations of 5 to 10 mg/l were effective against bacterial pathogens. Iodine was less dependent than chlorine upon the pH, temperature, contact time, and secondary reactions with nitrogenous impurities in the water. As a cysticide, iodine was poor in water with a high pH. Consequently, the tablet that was formulated contained an acid buffer to lower the pH of the water. The tablet, called "Globaline", released 8 mg/l of iodine, which is extremely high compared to the amount that is possibly needed for public water supplies. The tablet is not widely accepted since color, taste, and odor problems are fairly common (O'Connor and Kapoor, 1970).

Chambers _et_ _al_. (1952) investigated the effect of iodine concentration, pH, exposure time, and temperature on 13 enteric bacteria in a clean system. Their results are not completely quantitative in that the reported iodine concentration is that required to inactivate all test organisims plated out after 1, 2, and 5 minutes of contact. This was equivalent to approximately 99.9% activation with

TABLE 9

Comparative Values from Confirmed Experiments on Disinfecting Water with Iodine at 23°C to 30°C and pH 7.0[a]

Organism	Iodine mg/l	Minutes for 99% Inactivation	c.t[b]
Coliform bacteria	0.4	1	0.4
Poliovirus 1	20	1.5	30.0
f_2 Virus	10	3.0	30.0
Simian cysts	15	10.0	150.0

---

[a] References to these values appear in the text.

[b] Concentration of iodine times contact time.

the procedure that they followed. There was definitely an observed pH effect. At 2°C to 5°C some bacterial species required 3 to 4 times as much iodine for similar inactivation at pH 9.0 as was required at pH 7.5. For 5 minutes of exposure the required dosage for ≥99.9% bacterial inactivation in high pH water at 20°C - 26°C was always less than 1 mg/l. A summary of their work with _E_. _coli_ is given in Table 10. This is the best available information on iodine as a bactericide.

Efficacy Against Viruses

Studies on the efficacy of iodine on viruses have shown that viruses are more resistant to disinfection than are vegetative cells of bacteria. Krusé (1969) compared virus and bacterial inactivation. At an iodine dose of 10 mg/l in 0.048 mM potassium iodide at room temperature and pH 7.0, _E_. _coli_ was inactivated more rapidly than f2 virus but both were reduced by 4 logs in less than 1 minute. However, when the potassium iodide concentration was raised to 0.5M, bacterial inactivation was 4 logs in 1 minute while virus inactivation was only 0.5 log in 1 hour (Figure 6).

Berg _et_ _al_. (1964) measured the dynamics of survival for poliovirus, coxsackievirus, and echovirus that were iodinated at pH 6.0 and at temperatures of 5°C, 15°C, and 25°C. The work of the Johns Hopkins group from 1962 to 1969 (Krusé, 1969) was primarily with the virus model f2 although some comparative poliovirus 1 work was done. Cramer _et_ _al_.

TABLE 10

Concentrations of Iodine and Contact Times Necessary for 99% Inactivation of *Escherichia coli*[a]

Iodine, mg/1	Contact Time, min	c.t[b]	pH	Temperature, °C
1.3	1	1.3	6.5	2 - 5
0.9	2	1.8	6.5	
1.3	1	1.3	7.5	
0.7	2	1.4	7.5	
0.8	1	0.8	7.5	
0.6	2	1.2	7.5	
0.8	1	0.8	8.5	
0.9	2	1.8	8.5	
1.8	1	1.8	9.1	
1.2	2	2.4	9.1	
0.35	1	0.35	6.5	20 - 25
0.20	2	0.40	6.5	
0.45	1	0.20	7.5	
0.30	2	0.60	7.5	
0.45	1	0.45	8.5	
0.40	2	0.80	8.5	
0.45	1	0.20	9.1	
0.30	2	0.60	9.1	

---

[a] Data from Chambers *et al.*, 1952.

[b] Concentration of iodine times contact time.

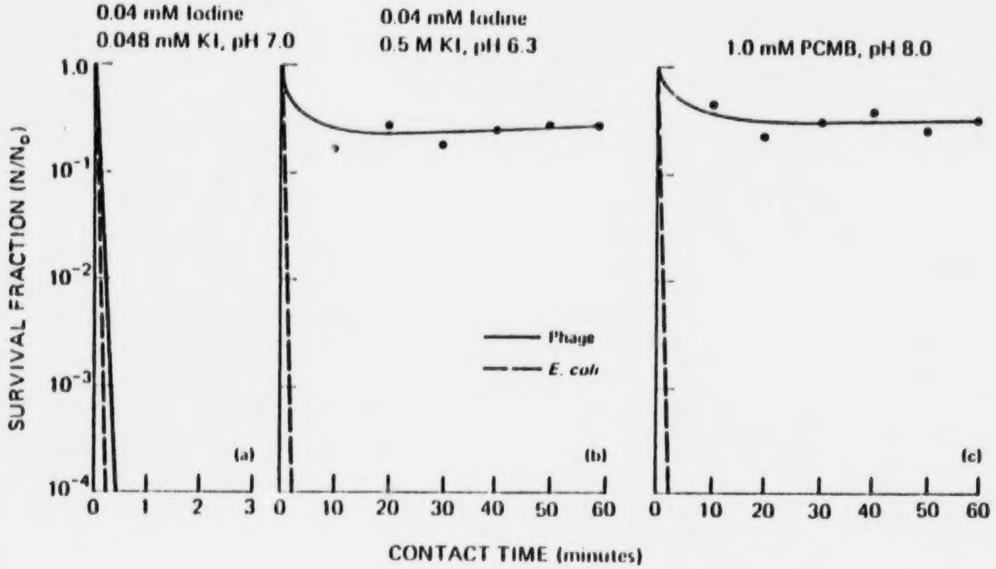

FIGURE 6

Survival of E. coli and f_2 bacteriophage reacted with 0.04 mM iodine containing (a) 0.048 mM potassium iodide (KI) at 37°C and (b) 0.5 M potassium iodide at 0°C, and survival obtained with (c) 1 mM of a sulfhydryl reacting agent, p-chloromercuribenzoic acid ($ClHgC_6H_4COOH$) From Kruse, 1969, with permission.

(1976) have shown that the mode of inactivation of these two viruses by iodine is similar. Data on the inactivation of virus by iodine is summarized in Table 11 for both polio and f2. The results of inactivation studies by the various groups compare very favorably. At pH 6.0 iodine approaches the order of magnitude of viricidal activity of hypochlorous acid (HOCl). At the pH's likely to be maintained in the distribution system (pH 8.0) iodine is a vastly more effective virucide than combined chlorine and is not far removed from the activity range of free chlorine.

Efficacy Against Parasites

Many studies on cyst inactivation have been reported, but there are discrepancies in their results due mainly to differences in the test systems that were used. The dose, pH, and temperature that were used in many of the studies are in doubt as are the different sources, cleaning methods for cysts, number of cysts used per test, and determination of viability. Stringer (1970) compared the resistance to iodine disinfection of Entamoeba histolytica cysts obtained from in-vitro culturing with those harvested from a human carrier and mixed amoebic cysts from monkeys. In water at room temperature at pH 6.5, three times the iodine dose was required in 10 minutes of exposure for 99% inactivation with wild (naturally formed) cysts compared to the cultured cysts. Large numbers of amoebic cysts from simian hosts

TABLE 11

Concentrations of Iodine and Contact Times Necessary for 99% Inactivation of Polio and f_2 Viruses with Flash Mixing

Test Microorganism	Iodine, mg/l	Contact Time, min	c.t[a]	pH	Temperature °C	References
f_2 Virus	13	10	130	4.0	5	Krusé, 1969
f_2 Virus	12	10	120	5.0		Krusé, 1969
f_2 Virus	7.5	10	75	6.0		Krusé, 1969
f_2 Virus	5	10	50	7.0		Krusé, 1969
f_2 Virus	3.3	10	33	8.0		Krusé, 1969
f_2 Virus	2.7	10	27	9.0		Krusé, 1969
f_2 Virus	2.5	10	25	10.0		Krusé, 1969
f_2 Virus	7.6	10	76	4.0	25 - 27	Krusé, 1969
Poliovirus 1	30	3	90	4.0		Krusé, 1969
f_2 Virus	64	10	64	5.0		Krusé, 1969
f_2 Virus	4.0	10	40	6.0		Krusé, 1969
Poliovirus 1	1.25	39	49	6.0		Berg *et al*., 196
Poliovirus 1	6.35	9	57	6.0		Berg *et al*., 196
Poliovirus 1	12.7	5	63	6.0		Berg *et al*., 196
Poliovirus 1	38	1.6	60	6.0		Berg *et al*., 196
Poliovirus 1	30	2.0	60	6.0		Cramer *et al*.,19
f_2 Virus	3.0	10	30	7.0		Krusé, 1969
Poliovirus 1	20	1.5	30	7.0		Krusé, 1969
f_2 Virus	2.5	10	25	8.0		Krusé, 1969
f_2 Virus	2.0	10	20	9.0		Krusé, 1969
f_2 Virus	1.5	10	15	10.0		Krusé, 1969
Poliovirus 1	30	0.5	15	10.0		Cramer *et al*.,19

---

[a] Concentration of iodine times contact time.

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were more readily available than cysts from human stools. They served as a reliable model for human E. histolytica.

The most extensive and reliable data on the cysticidal properties of iodine are found in the work of Chang and Stringer and their coworkers (Change and Baxter, 1955; Chang et al, 1955; Stringer, 1970; Stringer and Krusé, 1971; and Stringer et al., 1975). The only quantitative comparative study of the cysticidal properties of chlorine, bromine, and iodine believed to be published is that of Stringer, who reported a 99.9% inactivation. The earlier studies of others involved "total kills" and are quite dependent on the cyst density that was used.

All investigators more or less agree that iodine is an excellent cysticide in low pH waters (<pH 4.0) suggesting that molecular iodine (I_2) rather than hypoiodous acid, is the active agent. Chang (1958) observed that when the titratable iodine dosages exceed 20 mg/l in the presence of iodide ion, the triiodide ion (I_3^-) has a cysticidal efficiency that is equal to 1/11, 1/8, and 1/7 that of I_2 at 6°C, 25°C, and 35°C. He further calculated relative cysticidal efficiency of hypoiodous acid to be one-third that of I_2 at 6°C and one-half that of I_2 at 20°C.

There may be the problem of ineffective iodine residual in the distribution system where cross-connection introduction of cyst contamination is a possibility. This is due to the practice (for corrosion control) of maintaining water in the distribution system at approximately pH 8.0. At the low halogen residual levels that are usually maintained, the most cysticidal form, I_2, would be less prevalent than hypoiodous acid. Water at 25°C and pH 8.0 with a total iodine residual of 0.5, 1.0, and 2.0 mg/l would contain only 0.06, 0.2, and 0.62 mg/l of molecular iodine. Table 12 shows the time in minutes required for 90% inactivation of simian cysts with residuals of bromine, chlorine, and iodine in water at pH 6.0, 7.0, and 8.0 and a temperature of 30°C. At pH 8.0, iodine is inferior to free bromine and chlorine. However, in the presence of excess ammonia, with which the halogens could react, bromine appears to be more effective at pH 8.0 than do similar dosages of iodine or chlorine. Residual iodine in water at pH 8.0 has the ability to persist.

The kinetics of iodine as a cysticide in water at 20°C to 30°C have been assembled and compared. The cultured E. histolytica cyst data of Chang and coworkers (Chang and Baxter, 1955; Chang and Morris, 1953; Chang et al., 1955) and the results of Stringer et al. (1975) present a consistent picture. For cultured cysts in water at pH 5 the c.t values for 99% inactivation calculated by Chang were 200 at 3°C, 130 at 10°C, and 65 at 23°C (Chang and Morris, 1953). Stringer et al. (1975) extended the cultured cyst data at 30°C for a range of pH given below:

TABLE 12

Contact Times Necessary for Low Residual Bromine, Chlorine, and Iodine in Water at 30°C to Effect 99% Inactivation of Cysts from Simian Stools[a]

	Contact Time Required, min, by 10-Minute Halogen Residual Concentration, mg/l								
	0.5 mg/l			1.0 mg/l			2.0 mg/l		
pH	Br_2	Cl_2	I_2	Br_2	Cl_2	I_2	Br_2	Cl_2	I_2
	Buffered Water								
6.0	10	10	20	4	4	10	3	3	5
7.0	12	14	40	8	12	20	4	5	7
8.0	15	20	ND[b]	10	15	80	5	10	20
	Buffered Water in Presence of Excess Ammonia								
6.0	10	65	20	8	35	10	4	22	5
7.0	30	120	40	10	55	20	7	35	7
8.0	35	ND[b]	ND[b]	13	80	80	9	50	20

---

[a] Data from Stringer *et al.*, 1975. The proportions of the molecular species present in the test system depend upon the halogen, the pH, and the presence or absence of ammonia.

[b] ND = Not Determined.

pH	c.t for 99% inactivation
6.0	40
7.0	50
8.0	100
8.5	200

These c.t values from Stringer et al. (1975) were approximately one-half the values they obtained with E. histolytica cysts from human stools and for mixed cysts from simian stools.

Mechanism of Action

The failure of a strong commercial iodine disinfectant to inactivate poliovirus (Wallis et al., 1963) led to interest in the responsible mechanism, especially the role of pH and iodide ion. Berg et al. (1964) claimed that iodine inactivation of coxsackievirus resulted from biomolecular reaction with a single iodine molecule and that clumping of virions played a role in resistance to disinfection. Fraenkel-Conrat (1955) pointed out inconsistencies in the literature regarding the virucidal properties of iodine. Hsu (1964) and Hsu et al. (1965) clarified the mechanism of iodine on cells and virus. Hsu extracted fully active transforming DNA from iodine-inactivated Haemophilus parainfluenzae cells and just as much infectious RNA from f2 bacterial virus that had been inactivated (5 logs) by iodine as from noniodinated controls. Iodine inactivation, unlike the action of chlorine, appears to be attributable to a reaction with vital amino acids in proteins. Further experiments were conducted to determine whether sulfhydryl, tryptophanyl, histidyl, or tyrosyl groups were involved. With bacterial cells, there was a striking similarity between the kinetics of inactivation with iodine and the application of p-chloromercuribenzoic acid, a sulfhydryl reacting agent. While the active chemical species of iodine is not known, Hughes (1957), Allen and Keefer (1955), Bell and Gelles (1951), and Hsu (1964) hypothesized that the hydrated cationic iodine species (H_2OI^+) attacks the base, first against the sulfhydroxyl groups, and is not materially affected by the presence of iodide ion as was tyrosine. When sulfhydroxyl groups are the site of inactivation, a low pH should favor the reaction. With the viruses, the sulfhydroxyl group is not involved. Evidence of tyrosine's involvement came from parallel experiments showing similar patterns of inactivation curves between iodination of f2 virus and L-tyrosine. Li (1942, 1944) had shown that the presence of iodide ion and low pH iodine (I_2) inhibited the iodination of tyrosine. Both poliovirus and f2 virus inactivation with iodine was inhibited by the iodide ion (Cramer et al., 1976). At pH 4.0, both polio and f2 viruses survived iodination whereas at pH 10 inactivation was complete. Although iodine decomposes rapidly to iodate (IO_3^-) and iodide at pH 10, flash mixing of iodine (yielding hypoiodous acid) overcomes this difficulty effectively, and the virus inactivation is complete in less than 1 minute (Longley, 1964). Therefore, there is evidence that iodine action, with little or no iodide present, is effected by the modification of protein without destroying DNA or RNA (Brammer, 1963; Hsu, 1964). The mode of action of iodine in cyst penetration and inactivation has not been studied.

Conclusions

Iodine has many features that are comparable to free chlorine and bromine as a water disinfectant, but iodamines are not formed. Free iodine is an effective bactericide over a relatively wide range of pH. Field studies on small public water systems have shown that low levels of 0.5 to 1 mg/l of free iodine can be maintained in distribution systems and that the magnitude of residual is sufficient to produce safe drinking water with no adverse effects on human health. Like other halogens, the effectiveness of iodine against bacteria and cysts is significantly reduced by high pH, but unlike bromine and chlorine it is much more effective against viruses because of the enhanced iodination of tyrosine. Currently, its use is restricted primarily to emergency disinfection of field water supplies because of its high cost and because it is difficult to apply to large systems. The possible adverse health effects of increased iodide intake for susceptible individuals in the population must also be considered.

Research Recommendations

Studies should be conducted to determine the consequences for human health of the long-term consumption of iodine in drinking water with special regard for more suceptible subgroups of the population.

BROMINE

Chemistry of Bromine in Water

Bromine was first applied to water as a disinfectant in the form of liquid bromine (Br_2) (Henderson, 1935), but it can also be added as bromine chloride gas (BrCl) (Mills, 1975) or from a solid brominated ion exchange resin (Mills, 1969). Oxidation of bromide (Br^-) to bromine can also be accomplished either chemically or electrochemically. Oxidation with aqueous chlorine gives either bromine or hypobromous acid (HOBr) depending on the ratio of chlorine to bromide. Both bromine (Liebhafsky, 1934) and bromine chloride (Kanyaev and Shilov, 1940) hydrolyze to hypobromous acid:

$$Br_2 + H_2O \longrightarrow HOBr + H^+ + Br^- \qquad (21)$$

$$BrCl + H_2O \longrightarrow HOBr + H^+ + Cl^- \qquad (22)$$

Molecular bromine exists in water at moderately acid pH and high bromide concentrations since the equilibrium constant of Reaction 21 is 5.8×10^{-9} at 25°C. Like chlorine, bromine chloride has a much higher hydrolysis constant than this so it does not exist as the molecular form in appreciable concentrations under conditions of water treatment. The ratio of molecular bromine to hypobromous acid depends on both pH and bromide concentration. From the equilibrium expression for Reaction 21:

$$\log \frac{(Br_2)}{(HOBr)} = \log (Br^-) - pH + 8.24 \qquad (23)$$

Thus, for a solution containing 10 mg/l bromide and a pH of 6.3, 1% of the bromine is Br_2 while at lower bromide concentrations or higher pH aqueous bromine occurs almost entirely as hypobromous acid, which is a very weak acid with a dissociation constant (for Reaction 24) of 2×10^{-9} at 25°C (Farkas and Lewin, 1950).

$$HOBr \rightleftharpoons H^+ + OBr^- \qquad (24)$$

The hypobromite ion (OBr^-) becomes the major form of bromine above pH 8.7 at 25°C. Lower temperature decreases both of the above equilibrium values, thereby increasing the pH range where hypobromous acid is the major chemical form of bromine in water.

Bromine and bromine chloride also react with basic nitrogen compounds to form combined bromine or bromamines (Galal-Gorchev and Morris, 1965; Johannesson, 1958; Johnson and Overby, 1971):

$$HOBr + NH_3 \text{---}> NH_2Br + H_2O \qquad (25)$$

$$NH_2Br + HOBr \text{---}> NHBr_2 + H_2O \qquad (26)$$

$$NHBr_2 + HOBr \text{---}> NBr_3 + H_2O \qquad (27)$$

The observed breakpoint for ammonia (NH_3) solutions that have been treated with bromine is similar to that seen with chlorine. For bromine, this point corresponds to 17 mg/l bromine for 1 mg/l of ammonia nitrogen (NH_3-N). At this point, a minimum of bromamine stability occurs (Inman et al., 1976) as ammonia nitrogen is oxidized to nitrogen gas.

$$3HOBr + 2NH_3 \longrightarrow N_2 + 3HBr + 3H_2O \qquad (28)$$

At bromine to ammonia ratios higher than this and in the acid pH range, nitrogen tribromide (NBr_3) is stable. It is the most abundant bromamine in such aqueous solutions. At lower bromine concentrations, dibromamine ($NHBr_2$) can be present, but it is quite unstable. Only at alkaline pH values and very high ammonia concentrations such as those found in wastewater, are significant quantities of monobromamine (NH_2Br) formed. Organic bromamines are also formed, but there is little information on their forms or stability.

Production and Application

Bromine is produced by oxidation of bromide-rich brines (that contain between 0.05% and 0.6% bromide) with chlorine. Bromine is then stripped with steam or air and is collected as liquid Br_2.

Bromine chloride is produced by mixing equal molar quantities of pure bromine and chlorine (Mills, 1975). It condenses to liquid bromine chloride below 5°C at 1 atm pressure or above 30 psig at 25°C. In the liquid phase, more than 80% of the liquid is bromine chloride, and the remainder is Br_2 and Cl_2. In the gas phase, 40% of the bromine chloride dissociates to Br_2 and Cl_2 at 25°C.

Bromine has been applied to water as liquid Br_2. The difficulties that are encountered when handling the liquid and its corrosive nature, especially when wet, have encouraged the use of bromine chloride. Liquid bromine chloride is removed from cylinders under moderate pressure. It is then vaporized, and the gas is metered in equipment that is similar to that used for chlorine. Like chlorine, bromine chloride is shipped as the dry liquid in steel containers. Gas feeders must be made of Teflon, Kynar, or Viton plastics because bromine chloride is more reactive than chlorine with polyvinylchloride plastics.

Analytical Methods

Bromine concentrations can be measured iodometrically by procedures that are identical to those used to measure total chlorine residuals (Standard Methods, 1976). None of these procedures is capable of distinguishing free bromine (Br_2, HOBr, OBr^-) from combined bromine (bromamines) or other oxidants that are capable of reacting with iodide under the slightly acid conditions used in these procedures. However, bromate does not interfere except at low pH (Kolthoff and Belcher, 1957). UV spectroscopy can be used to measure the bromamines selectively in the presence of one another and without interference from free bromine because none of the free bromine forms except Br_3^- has strong UV absorptivities (Johnson and Overby, 1971).

Biocidal Activity

As residual bromine was rarely measured in the studies cited here c.t (concentration, mg/l, times contact time, min) products have generally been calculated using the dosage of bromine added to the system.

Efficacy Against Bacteria

The efficacy of bromine inactivation of bacteria has been summarized by Farkas-Hinsley (1966). Vegetative cells are readily inactivated by bromine but reports often leave the type of bromine compound and its residual concentration as uncertain. Consequently, it is difficult to make quantitative comparisons with other disinfectants. Tanner and Pitner (1939) reported that the concentration of hypobromous acid that are required to give "complete kill" in 30 minutes are 0.15 mg/l for _Escherichia_ _coli_ and 0.6 mg/l for _Salmonella_ _typhi_. Spores of _Bacillus_ _subtilis_ required more than 150 mg/l of bromine. Krusé _et_ _al_. (1970) found that 4 mg of bromine per liter of hypobromous acid gave 5 logs of disinfection of _E_. _coli_ in 10 minutes at 0°C. At pH 4.5, where significant Br_2 was present, 2 mg/l bromine at 0°C gave 4 logs of _E_. _coli_ disinfection in 3 minutes. However, Kruse also reported that 0.1 M bromide decreased _E_.

coli disinfection to 2 logs of inactivation from 4.5 logs at 0.001 M bromide, using 4 mg/l bromine at pH 7.5 and 0°C in each case. This is in conflict with observations made at low pH since high bromide should also produce bromine. The data at pH 4.5 showed 4 logs inactivation with a c.t of 6 compared to 5 logs inactivation from a c.t of 40 for hypobromous acid. Even these data, the best available, are based on the concentrations of bromine added to the solution. It is difficult to compare the various studies on disinfection by bromine since the chemical species of bromine present in most instances ave not been measured or reported.

The effect of the formation and decomposition of the bromamines on the efficacy of bacterial disinfection was first discussed by Johannesson (1958). He reported that 0.28 mg/l of monobromamine expressed as bromine resulted in 99% inactivation in less than 1 minute, while the same concentration of N-bromodimethylamine [$N(CH_3)_2Br$] required 12 minutes for 99% inactivation of E. coli. The measurements of the halogen remaining after the experiments showed very little loss.

The efficacy of bromine inactivation of spores of Bacillus metiens and B. subtilis has been studied by Marks and Strandskov (1950) and Wyss and Stockton (1947). Both pairs of investigators found that the activity was markedly pH-dependent at low pH values where molecular bromine predominates. The activity increased rapidly from pH 4 to pH 3, the lowest that was tested. The c.t required for 99% inactivation was 20-25 at 25°C and pH 3.0. At pH 6 to 8, the c.t was 225 to 375 (Wyss and Stockton, 1947). At pH 9, where the hypobromite ion starts to predominate, the c.t increased to over 500.

Marks and Strandskov (1950) also determined that at pH 7 and 25°C monobromamine was one-half as effective as hypobromous acid against B. metiens spores. N-bromosuccinimide was 1/17, and N-bromopiperidine was 1/800 as effective. Wyss and Stockton (1947) showed the effect of changing chemical form and concentration by increasing the concentration of ammonia that was added to a 20 mg/l bromine solution at pH 7 containing B. subtilis spores. Table 13 shows the measured residual at the time required for 99% inactivation. Before the ammonia is added to the solution, hypobromous acid is the major chemical form. When the ammonia nitrogen reaches 1 mg/l, nitrogen tribromide predominates. At 2 mg/l ammonia nitrogen, the breakpoint occurs with rapid loss of combined bromine residual. At 10 and 30 mg/l ammonia nitrogen, dibromamine is present and is less stable and effective than hypobromous acid. At 1,000 mg/l ammonia nitrogen, monobromamine has a c.t value for 99% inactivation that is as effective as nitrogen tribromide and hypobromous acid.

The effect of temperature on the efficacy of hypobromous acid inactivation of B. subtilis spores is shown in Table 14 (Wyss and Stockton, 1947). The activity increases

TABLE 13

The Effect of Ammonia on Bromine Concentrations Required for 99% Inactivation of Bacillus subtillis Spores at 20 mg/1, pH 7, 25°C[a]

Ammonia, mg NH_3-N/1	10-min residual,[b] mg/1	Contact time, min	c.t[c]
0	20	14	280
1	16	19	304
3	2	>100	>200
10	8	85	680
30	9	70	630
100	14	18	252
1,000	19	14	266

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[a] Data from Wyss and Stockton, 1947.

[b] Initial bromine concentration 20 mg/1.

[c] Concentration of bromine times contact time.

TABLE 14

The Effect of Temperature on Bromine Concentrations Required for 99% Inactivation of *Bacillus subtilis* Spores with 25 mg/l Bromine at pH 7.0[a]

Temperature, °C	Contact Time, min	c.t[b]
35	4	100
25	10	250
20	16	400
15	21	525
10	37	925
5	54	350

[a] Data from Wyss and Stockton, 1947.

[b] Concentration of bromine times contact time.

approximately 2.3 times for each 10°C rise in temperature. After measuring washed vegetative cells of *B. subtilis*, Wyss and Stockton also found them to be 500 times less resistant. They reported no temperature data for their vegetative cell studies.

Efficacy Against Virus

Krusé *et al*. (1970) reported that 4 mg/l of bromine as hypobromous acid at pH 7 and 0°C gave 3.7 logs of inactivation of f2 *E. coli* phage virus in 10 minutes. At higher bromide concentrations and lower pH, where Br_2 becomes the principal form of bromine, the rates of inactivation increased. A c.t of 40 was required for 3.7 logs inactivation at pH 7.0 and 0°C compared to a c.t of 1 for 4.5 logs inactivation at 0°C and pH 4.8. At pH 7.5 and with a concentration of 0.1 M bromide at 0°C, a c.t of 12 yielded 5.5 logs inactivation. In these studies the residual concentrations of bromine were probably not significantly different from those in solutions that did not contain added salts. In the presence of added bromide, amines were difficult to quantify. After 2 hours, Krusé and his colleagues found no viable virus in a solution of 4 mg/l bromine, starting from an initial titre of 1.7×10^9 plaque-forming units (PFU)/ml at 0°C and pH 7.5, when excess ammonia was present. However, the addition of bromine to phage solutions that contained excess methylamine (CH_3NH_2) and glycine (H_2NCH_2COOH) essentially stopped disinfection under these conditions. This may be due either to the formation of the N-bromo compounds or to the reduction of bromine.

Taylor and Johnson (1974) used Φx174 E. coli phage and measured concentrations of the disinfecting species in constant residual solutions. They reported that 0.32 mg/l hypobromous acid required only 1.1 minutes or a c.t of 0.35 to inactivate 99% of this phage at 0°C. Compared to hypobromous acid, molecular bromine was approximately three times as fast, requiring Br_2 at c.t of 0.1. Nitrogen tribromide was less potent requiring a c.t of 1.0 for 99% inactivation of this phage at 0°C.

The effect of temperature on Φx174 inactivation was measured for 0.16 mg/l of hypobromous acid expressed as bromine at pH 7. The Arrhenius plot of ln k against 1/T (where T is temperature) from 273°K to 303°K gave a slope equivalent to 37 Kcal/mole or an increase in rate of inactivation of 1.9 times for a rise in temperature from 15°C to 25°C.

Sharp's group (Floyd et al., 1976, 1978; Sharp et al., 1975, 1976) have made careful studies with measured, controlled residual concentrations. They demonstrated that even under these controlled chemical conditions the degree of aggregation or clumping had a marked effect on the apparent, observed inactivation rates with reovirus and type 1 poliovirus (Sharp et al., 1975). Reoviruses, as single particles, required only 1 second for 3 logs of inactivation at pH 7 and 2°C with hypobromous acid at 0.46 mg/l bromine. Aggregated samples gave much slower rates, especially at high levels of inactivation. At 3 logs of inactivation, the time required doubled for the same concentration conditions (Sharp et al., 1976). Poliovirus 1 was also rapidly inactivated by hypobromous acid when single virus particles were studied (Floyd et al., 1976). At 2°C and pH 7, only 3.5 mg/l bromine or a c.t of 0.2 was required for 2 logs of inactivation. However, the rate of inactivation did not increase linearly with concentration at this temperature. Higher concentrations were much less efficient than longer exposures. At 1 mg/l hypobromous acid, 7 seconds were required for 1 log of inactivation or a c.t of 0.23 for 2 logs of inactivation at 2°C and pH 7 with hypobromous acid. The inactivation rate increased only slightly with temperature at this concentration. At 10°C a c.t of 0.21 for 2 logs of inactivation and at 20°C a c.t of 0.06 was required for 99% inactivation with hypobromous acid.

The inactivation of single poliovirus particles in buffered, distilled water and constant residual concentrations for the other major bromine chemical forms have also been studied by Floyd et al. (1978). Table 15 gives the calculated c.t required to yield 99% inactivation for these different forms near 1 mg/l expressed as bromine. The values in Table 15 depend on concentration except for Br_2, for which time and concentration were inversely related as normally assumed from the Watson-Chick relationship

(Morris, 1975). Floyd et al. (1978) also demonstrated that dibromamine, nitrogen tribromide, and hypobromous acid were less efficient at higher concentrations while the hypobromite ion became more effective as the concentration increased. The rate for the hypobromite ion is rapid compared to the other bromine compounds. This is interesting in light of the fact the hypochlorite ion (OCl^-) is generally considered to be a much poorer disinfectant than hypochlorous acid (HOCl).

Efficacy Against Parasites

There appears to be no information regarding the effectiveness of bromine as a disinfectant against eggs or larvae of helminth parasites in water.

With protozoa that are important to public health, attention appears to have been devoted primarily to the efficacy of bromine in destroying cysts of Entamoeba histolytica. Most relevant information has been provided by Stringer et al. (1975) as a result of their studies on the comparative cysticidal efficacies of various halogens (shown in Table 12 in the section on Iodine). They used bromine stock solutions that were prepared by bubbling nitrogen through bottles of elemental bromine. Concentrations were determined by a colorimetric bromocresol purple test. Cyst survival was evaluated by the excystation method that these investigators had developed.

TABLE 15

Exposure (c.t)[a] to Various Bromine Compounds Required for 99% Inactivation of Poliovirus 1, Mahoney[b]

Chemical Form	c.t	Temperature, °C	pH
Dibromamine ($NHBr_2$)	1.2	4	7.0
Nitrogen tribromide (NBr_3)	0.19	5	7.0
Bromine (Br_2)	0.03	4	5.0
Hypobromite ion (OBr^-)	0.01	2	10.0
Hypobromous acid ($HOBr$)	0.24	2	7.0
Hypobromous acid	0.21	10	7.0
Hypobromous acid	0.06	20	7.0

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[a] Concentration of compound times contact time.

[b] Data from Floyd et al., 1978.

In dose-response experiments in distilled water for exposures of 10 minutes and at pH levels from pH 4 to pH 10, bromine was found by Stringer et al. (1975) to be the most effective and fastest acting of the halogens tested over the widest pH range. At pH 4, 99.9% cyst mortality was obtained with 1.5 mg/l of free bromine residual; whereas, 2 mg/l of chlorine and 5 mg/l of iodine were required to attain the same mortality. Furthermore, increases in pH seemed to have less effect on the cysticidal efficacy of bromine as compared with the other halogens. At pH 10, 99.9% mortality was obtained with residuals of 4 mg/l of bromine, 12 mg/l of chlorine, and 20 mg/l of iodine.

In studies more nearly simulating usual water treatment procedures, "flash mixing" of halogens at a dosage of 2 mg/l with the cyst suspensions was used. In buffered distilled water, bromine again proved to be the most effective: 99.9% inactivation was obtained at pH 6 and pH 8 after 15 minutes of contact. Longer exposures at the lower pH were required for the other halogens in order to provide even less inactivation than this.

An interesting aspect of the study of Stringer et al. (1975) was that ammonia bromamines were nearly as cysticidal as free bromine excpet in waters of high pH (Table 12).

Thus, under conditions likely to be found during the treatment of natural water supplies, free and combined bromine appear to be a practical, effective cystide, at least as far as cysts of E. histolytica are concerned.

Mechanisms of Action

The pattern of bromine disinfection appears to be similar to that of chlorine. After comparing the activity of chlorine, bromine, and iodine against spores, Marks and Strandskov (1950) noted that Br_2 was nine times more effective than hypobromous acid and that the hypobromite ion and tribromide ion (Br_3^-) had very low activity. They noted that a high degree of polarity contributed to the inactivity of the ionic forms and the reduced activity of the hypohalous acid compared to the free molecular halogen. They also found that "the killing rates of bacterial spores for the hypohalous acids and probably for the molecular halogens decrease in the [following] order: chlorine, bromine, iodine."

Many workers attribute this decrease to the greater oxidation potential of the halogens with lower molecular weight. However, the polarity and perhaps the sizes of the halogens may be important in getting the disinfectants to the vital site.

Olivieri _et al_. (1975) demonstrated that bromine was effective in inactivating both naked viral RNA and intact virus. The primary site of inactivation of f2 phage more likely involves the reaction of bromine with the protein coat of the virus, because inactivation of RNA that was prepared from bromine-treated virus lagged significantly behind the inactivation of intact virus. The mechanism of inactivation with bromine, as with the other halogens, involves moving the disinfectant to the vital site, mass transport, and the reactivity of the bromine with that site, oxidation. The effectiveness of bromamines as disinfectants may be explained by their relatively low polarity and high reactivity. The effectiveness of the hypobromite ion as a virucide may be due to the fact the high pH's of the solutions make the virus more sensitive to the ion. Or, it could be attributed to Olivieri's observation that bromine acts primarily on the protein coat of viruses.

Summary and Conclusions

Laboratory tests show that bromine is an effective bactericide and virucide. It is more effective than chlorine in the presence of ammonia. As a cysticide it is highly active. Bromine is active over a relatively wide range of pH, and it retains some of its effectiveness as hypobromous acid to above pH 9.

The major disadvantage of bromine as a disinfectant is its reactivity with ammonia or other amines which may seriously limit its effectiveness under conditions that are encountered in the treatment of drining water. Data on the effectiveness of bromine against bacteria are complicated by this reactivity and the lack of characterization of the residual species in disinfection studies.

Research Recommendations

Further research is required to quantitate the effectiveness of the various species of bromine and the bromamines, both organic and inorganic, against bacteria, viruses, and cysts, particularly with bacteria.

The reactivity of bromine and the doseages that are required to maintain effective residual in natural water systems should be investigated.

The effectiveness of various technologies for application of bromine or bromine chloride to intended drinking water should be evaluated.

FERRATE

Ferrates are salts of ferric acid (H_2FeO_4) in which iron is hexavalent. Fremy (1841) first synthesized potassium ferrate (K_2FeO_4) in the mid-19th century. Since then, a wide variety of metallic salts have been prepared. However, only a few of the preparations yield ferrates of sufficient purity and stability for use in the treatment of water. Ferrates are strong oxidizing agents that have a redox potential of -2.2V or 0.7V in acid and base, respectively (Wood, 1958).

The Chemistry of Ferrate in Water

Aqueous solutions of potassium ferrate are unstable and decompose to yield oxygen (O_2), hydroxide (OH^-), and insoluble hydrous iron oxide [FeO(OH)]:

$$2FeO_4^{2-} + 3H_2O \dashrightarrow 2FeO(OH) + \frac{3}{2}O_2 + 4OH^- \qquad (29)$$

The hydrous iron oxide is a coagulant that is commonly used in water treatment. Initial ferrate concentration, pH, temperature, and the surface character of the resulting hydrous iron oxide affect the rate of decomposition (Schreyer and Ockerman, 1951; Wagner et al., 1952; Wood, 1958). Ferrate solutions are most stable in strong base (>3M or at pH 10 to 11). Schreyer and Ockerman (1951) reported that dilute aqueous solutions of ferrate are more stable than concentrated solutions. The presence of other inorganic ions also affect ferrate stability in aqueous solutions.

Ferrate reacts rapidly with reducing agents in solutions (Murmann, 1974). It will also oxidize ammonia (NH_3). The rates for this reaction increase with pH (optimum pH range 9.5 - 11.2), molar ratio of ferrate to ammonia, and temperature (Strong, 1973). The oxidation of ammonia by ferrate below pH 9 is markedly slower than that observed for chlorine. Some information is available on the reaction of ferrates with various organic compounds (Audette et al., 1971; Becarud, 1966; Zhdanov and Pustovarova, 1967).

Preparation

Concentrated solutions of sodium ferrate (Na_2FeO_4) may be prepared electrochemically from the more common iron forms. Scrap iron can be converted to ferrate iron with a 40% efficiency (Murmann, 1974). Potassium ferrate may be prepared by wet oxidation of Fe(III) with potassium permanganate ($KMnO_4$). Subsequent recrystallization yields a crystalline solid with greater than 90% purity (Schreyer et al., 1950). Ferrate salts are not commercially available. Consequently, sufficient quantities for pilot or full-scale testing would have to be especially prepared.

Analytical Methods and Residuals

Aqueous ferrate solutions have a characteristic violet color that is similar to permanganate and a wavelength maximum at 505 nm in the visible portion of the electromagnetic spectrum. The molar extinction coefficient at 505 nm in 10^{-4} M sodium hydroxide (NaOH) was 1,070 ± 30 $1M^{-7}$ cm^{-1} (Wood, 1958). Since ferrates are unstable, residual cannot be maintained.

Biocidal Activity

Efficacy Against Bacteria

Gilbert *et al.* (1976) reported the inactivation of a pure culture of *Escherichia coli* at pH 8.0. Increased ferrate concentrations from 1.2 to 6.0 mg/l yielded increased rates of inactivtion of *E. coli*. They observed 99% inactivation at 6.0 mg/l, pH 8.0, and 27°C in approximately 8.5 minutes. The rates of inactivation of *E. coli* with ferrate appear to be of the same order of magnitude as monochloramine (NH_2Cl). Waite (1978a, b) extended Gilbert's disinfection studies to include enteric pathogens and Gram-positive bacteria, and evaluated the effects of pH and temperature. The rate of *E. coli* inactivation by ferrate increases as pH decreases from pH 8.0 to pH 6.0. At the lower pH value, the rate of ferrate decomposition is increased, and little inactivation was observed after 5 minutes. Several inconsistencies at low ferrate concentrations (0.12 mg/l) were observed, but low-level inactivations (<50% inactivation) are difficult to interpret. At lower temperatures, the biocidal activities of ferrate appears to increase as temperature increases. At higher temperatures, the ferrate decomposition becomes an important factor. _Salmonella typhimurium_ and _Shigella flexneri_ were inactivated in a manner similar to that of _E. coli_. However, considerably higher dosages of ferrate (>12 mg/l) were necessary for inactivation of _Streptococcus faecalis_. Ferrate concentrations of 12 mg/l and 60 mg/l required 5 minutes and 15 minutes, respectively, for 99% inactivation at pH 8.0. Other gram-positive bacteria tested (_Bacillus cereus_, _Streptococcus bovis_, and _Staphylococcus aureus_) were noticeably more resistant to ferrates than were the enteric bacteria. Higher doses of ferrate (15-20 mg/l) were also necessary to inactivate 99% to 99.9% of the bacteria in the presence of organic material in wastewater.

Efficacy Against Viruses

Waite (1978a, b) reported that the inactivation of the RNA bacterial f2 virus with ferrate appeared to be more dependent on pH than was bacterial inactivation. Considerably more viral inactivation was observed at pH 6.0 than at pH 8.0. At 1.2 mg/l ferrate, just under 4 minutes were required for 99% inactivation, while for similar conditions 13 minutes were necessary at pH 8.0.

The disinfection efficacy of ferrate is summarized in Table 16. The ferrate dose is given in the table since ferrate residuals were not reported. Ferrate decomposes rapidly in aqueous solution, and the dose represents an overestimate of ferrate concentration. Nevertheless, the c.t products (concentration, mg/l, times contact time, min) indicates the difference in efficacy relative to the test microorganism and provide a crude idea of the c.t product for ferrate.

Mechanism of Action

No studies have elucidated the mechanism of inactivation caused by ferrate.

Conclusions

While not as biocidal as the free halogens, chlorine dioxide, or ozone, ferrate appears to be similar to or slightly better than the chloramines as a bactericide and more active as a virucide than the chloramines. The combined application of ferrate as a disinfectant and a coagulant makes it an attractive alternate biocide. Far more information on the feasibility of large-scale preparation of ferrate and its biocidal activity (particularly under water-treatment conditions) are necessary before ferrate can be given consideration as a water disinfectant for public systems.

TABLE 16

Concentrations of Ferrate (FeO_4^{2-}) and Contact Times Necessary for 99% Inactivation of *Escherichia coli*, *Streptococcus faecalis*, and f_2 virus[a]

Test Microorganism	Ferrate Dosage mg/l	Contact Time, min	c.t[b]	pH	Temperature, °C
Escherichia coli	1.2	4.6	5.52	7.0	20
	6.0	2.5	15.0		
Streptococcus faecalis	1.2	365	438	7.0	20
	6.0	4.0	24		
f_2 virus	1.2	3.7	4.4	6.0	20
	6.0	1.5	9		

[a] Data from Waite, 1978.

[b] Concentration of ferrate times contact time.

HIGH pH CONDITIONS

High pH values are obtained when drinking water is softened by the commonly used precipitation method which removes calcium (Ca^{+2}) and magnesium (Mg^{+2}) ions that cause water hardness. In such cases, calcium hydroxide [$Ca(OH)_2$] is the usual source of hydroxide that is used to raise the pH. In this process, pH values as high as 10.5 are reached and maintained up to 6 hours. Low pH conditions are not used in water treatment processes. Therefore, while low pH's are lethal to most microorganisms, they are of less interest in this report than high pH conditions which might have potential for disinfection through modification of current practices.

Attainment of Elevated pH

High pH values in water are obtained by using either calcium hydroxide or sodium hydroxide ($NaOH$). Sodium hydroxide is a by-product of the production of chlorine by the electrolysis method. Calcium hydroxide is prepared by reacting calcium oxide (CaO) with water. The calcium oxide is produced by heating calcium carbonate ($CaCO_3$) to drive off the carbon dioxide (CO_2). The calcium oxide is normally prepared off the site of the water treatment plant.

However, it is often slaked on site at all but the smallest plants, which purchase calcium hydroxide and use it directly. A few, very large water treatment plants produce their own calcium hydroxide by calcining their water-softening sludge.

Hydroxide (OH$^-$) is added to water as a water slurry containing calcium hydroxide or as a water solution of sodium hydroxide. In the usual water-softening technique, the water is flocculated for 10 to 30 minutes to promote the precipitation reactions. The precipitates are then removed by sedimentation with retention times from 1 to 6 hours.

Disinfection with high pH is readily accomplished, but the required pH values are so high that the pH must be reduced before the water is consumed. In a full-scale water treatment plant, this is accomplished in the recarbonation stage, which is a normal component of the water-softening process. However, for small institutions or private residences the pH reduction requirement would mitigate against the use of this disinfection technique.

Analytical Methods and Process Control

The pH can be reliably measured with the glass electrode at pH values of approximately 7 and at room temperatures. Special care must be taken to obtain accurate values at other temperatures and at the elevated pH values that are necessary for disinfection with this process.

Residual

The hydroxide that is used to obtain the necessary pH values enters into few side reactions in natural water after the initial formation of metallic hydroxides and the reactions with carbon dioxide. The amounts of hydroxide for these reactions can be readily computed after the water has been chemically analyzed. There is a slow additional loss of hydroxide from the water by reaction with carbon dioxide entering from the atmosphere. However, this is not a serious problem.

Biocidal Activity

The microbial inactivation under consideration in this report is that caused solely by high pH. When the pH of water is raised, the opportunity exists for precipitation of many compunds associated with carbonates, oxides, and hydroxides, among many others. Even in distilled water, calcium carbonate may precipitate if calcium hydroxide is used to raise the pH and if sufficient carbon dioxide enters the water during the experiment. These precipitates provide opportunities for adsorption and coagulation of microorganisms and will cause an increased removal over that obtained from the pH effect alone without the precipitate. The research cited below, unless otherwise noted, was conducted with procedures that obviated this problem or that made its effect insignificant.

Efficacy Against Bacteria

Wattie and Chambers (1943) determined the times required to obtain 100% inactivation of several bacterial species at initial concentrations of approximately 1,500 organisms/ml in 20°C to 25°C dechlorinated tapwater, using calcium hydroxide for pH adjustment. (See Table 17.) The inactivation was significantly less at each pH for each organism between 0°C to 1°C. At a pH of 11.5, the time for 100% inactivation of *Escherichia coli* was increased from 210 minutes between 20°C to 25°C to 355 minutes between 0°C to 1°C. The time for 100% inactivation of *Salmonella typhi* was increased from 75 minutes to 270 minutes for the same temperature change. No mention was made of difficulties from the formation of precipitates.

Riehl *et al*. (1952) reported *E. coli* inactivations of 95% in 8 hours at pH 10.5 and 5°C, 100% in 2 hours at pH 10.5 and 15°C, and 100% in approximately 30 minutes at pH 10.5 and 25°C in distilled water. Calcium hydroxide was used for pH adjustment. Inactivations of 50% and 55% in 10 hours were noted for *Salmonella montevideo* and *S. typhi*, respectively, at 2°C and pH 10.6 in distilled water. Initial concentrations of organisms were approximately 1,000/ml. They also observed that higher temperatures gave more inactivation at the same pH and contact time and that the composition of the water did not markedly influence the bacterial survival.

TABLE 17

Contact Times Necessary for High pH Conditions to Effect 100% Inactivation of Initial Concentrations at 1,500 Organisms/ml[a]

	Contact Time Required for 100% Inactivation by High pH Conditions, mi				
pH	Escherichia coli	Enterobacter aerogenes	Pseudomonas aeruginosa	Salmonella typhi	Shigella dysenteriae
9.01 - 9.5	>540	--	--	>540	--
9.51 - 10.0	>600	>600	420	>540	>300
10.01 - 10.5	>600	>600	300	>540	>300
10.51 - 11.0	600	>540	240	240	180
11.01 - 11.5	300	>600	120	120	75

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[a] Data from Wattie and Chambers, 1963. Experiments were conducted at 20°C - 25°C.

Berg and Berman (1967) determined the inactivation of E. coli at 25°C in the dilution water medium that is described in the 11th edition of Standard Methods (1960) (see Table 18). Sodium hydroxide was used to adjust the pH.

Efficacy Against Viruses

Wentworth et al. (1968) reported that little or no poliovius 1 was inactivated in distilled water at pH 11.2 at room temperature in 90 minutes when calcium hydroxide or sodium hydroxide was used to increase the pH. For poliovirus 1 in distilled water without added salts, Sproul et al. (1970) showed that no inactivation occurred in 90 minutes at pH values of 10.5 or less when calcium hydroxide and sodium hydroxide furnished the hydroxide. Their work was done at 21°C to 22°C. Sproul (1975) obtained poliovirus 1 inactivations in 30 minutes of 7% at pH 11.5, 59% at pH 11.9 in 30 minutes, 94% at pH 12.1 in 20 minutes, and 99.83% at pH 12.5 in 5 minutes. He used distilled water with 100 mg/l of sodium chloride (NaCl) at 22°C to 23°C.

Berg and Berman (1967) showed the inactivation of Echovirus 7 A3KP8A1 at 25°C in a dilution water medium (Standard Methods, 1960). They used sodium hydroxide to adjust pH. (See Table 19.)

From his work with the f2 bacteriophage, Donovan (1972) suggested that a calcium-virus complex was formed and that a large part of the observed decrease in titer at pH 11.5 was

TABLE 18

Inactivation of Escherichia coli by High pH Conditions at 25°C in a Dilution Medium[a]

pH	Contact time, min	Inactivation %
11.55	50	~10
11.70	50	~90
11.81	35	96
12.01	10	99.8
12.04	6	97

[a] Data from Berg and Berman, 1967.

TABLE 19

Inactivation of Echovirus 7 by High pH Conditions at 25°C in a Dilution Medium[a]

pH	Contact time, min	Inactivation %
11.23	7	99.98
11.49	4	99.99
11.79	4	99.5
11.92	1.5	99.9

---

[a] Data from Berg and Berman, 1967.

caused by an aggregation of the virus. Electron micrographs and chemical evidence supported the observation.

Efficacy Against Parasites

The susceptibility of protozoa or helminths to inactivation by high pH conditions does not appear to have been studied.

Mechanisms of Action

The inactivation mechanism of high pH on bacteria has not been examined. There is ample evidence to show that the poliovirus is inactivated by the disruption of the capsid and a loss of the RNA to the water (Boeye and Van Elsen, 1967; Maizel _et al_., 1967; Van Elsen and Boeye, 1966). Boeye and Elsen (1967) suggested that at pH 10 and at elevated temperatures (≥30°C), the RNA was released from the capsid in a degraded form or that it was quickly and extensively broken down after release. The applicability of this mechanism to enteroviruses other than poliovirus is unknown.

Conclusions

The utilization of high pH conditions for disinfection of water is feasible, but higher pH values than are normally used in water treatment and long contact times are required. At pH values of approximately 10.5, bacteria are inactivated in up to 600 minutes, but viruses are probably unaffected. A pH value of 12 to 12.5 and a contact time of approximately 30 minutes can probably yield 99% to 99.9% inactivation of most bacteria and of certain viruses. This process has a drawback: the pH that is necessary for effective disinfection must be reduced before the water can be consumed. Use of the high pH as the sole means of disinfection is not recommended. In many situations where water is softened by the precipitation process and where the water is of poor biological quality, the disinfection potential of this process could be used by increasing the pH above its present values.

Research Recommendations

While this method has not been used deliberately for disinfection in the past since high pH conditions are attained in many treatment plants, additional work on this method may be warranted. Studies are needed to determine the pH values required for inactivation of protozoans, to determine the pH values necessary for inactivation of a broader and more representative group of viruses, and to determine the pH values required to inactivate a larger group of enteric bacteria.

HYDROGEN PEROXIDE

Hydrogen peroxide (H_2O_2) is a strong oxidizing agent that has been used for disinfection for more than a century. Its instability and the difficulty of preparing concentrated solutions has tended to limit its use. However, by 1950 electrochemical and other processes were developed to produce pure hydrogen peroxide in high concentration, which is known as stabilized hydrogen peroxide (Schumb et al., 1955). This product has been subjected to increased study and application. Most recently it has been used to disinfect spacecraft (Wardle and Renninger, 1975), foods (Toledo, 1975), and contact lenses (Gasset et al., 1975; Spaulding et al., 1977). Although there has been some interest in using hydrogen peroxide as a disinfectant for wastewater (Taki and Hashimoto, 1977), it has been used more for control of bulking in the activated sludge waste-treatment process (Sezgin et al., 1978). Its use in drinking water disinfection appears minimal.

Analytical Methods

Analytical methods for hydrogen peroxide are based on oxidation or reduction with potassium permanganate ($KMnO_4$), potassium iodide (KI), or ceric sulfate [$Ce(SO_4)_2$] (Chadwick and Hoh, 1966). A colorimetric procedure based on the oxidation of titanium sulfate ($TiOSO_4$) (Snell and Snell,

1949) is sensitive to about 1 mg/l in the absence of other oxidizing agents.

Production and Application

Commercially produced hydrogen peroxide is available in aqueous solution, usually ranging from 30% to 90%. It is stabilized during manufacture by addition of such compounds as sodium pyrophosphate ($Na_4P_2O_7$), acetophenetidin ($CH_3CONHC_6H_4OC_2H_5$), or acetanilide ($CH_3CONHC_6H_5$). As the concentration of hydrogen peroxide is decreased, the concentration of stabilizers is typically increased. Experience in the waterworks industry using hydrogen peroxide is nonexistent. However, hydrogen peroxide is widely used as a bleaching agent in making cotton textiles or in wood pulping (Chadwick and Hoh, 1966). Presumably, a concentrated solution would be diluted and applied with a chemical metering pump. Careful attention to safety in handling would be required because of the possibility of fire or explosion.

Biocidal Activity

The few pre-1965 references on disinfection by hydrogen peroxide have been summarized by Yoshpe-Purer and Eylan (1968). Although its bactericidal activity was indicated, a need for catalytic Fe^{++} or Cu^{++} was reported. Yoshpe-Purer and Eylan (1968) worked with _Escherichia_ _coli_, _Salmonella_ typhi, and Staphylococcus aureus in pure culture and as a mixture of bacteria. They also studied the effect of hydrogen peroxide concentrations (from 30 to 60 mg/l), contact time (10 to 420 minutes), and initial concentration of organisms. Without ever measuring residuals, they concluded that bacterial inactivations were relatively slow, that E. coli was more resistant to hydrogen peroxide than S. typhi or S. aureus, and that the required inactivation time was increased as the initial concentration of organisms was increased. Regardless of the hydrogen peroxide concentration or the type of organism used , all tests were claimed to be "sterile" in 24 hours. Table 20 summarizes some of these data.

Toledo et al. (1973) studied inactivation of S. aureus and spores of several species of Bacillus and Clostridium with 25.8% (258,000 mg/l) hydrogen peroxide. The time to reduce S. aureus by 6 logs was 1 minute, whereas reduction of spores by 99% required from less than 1 minute to approximately 17 minutes, depending on the species. Increasing the hydrogen peroxide concentration up to 41% (or 410,000 mg/l) or increasing temperature from 24°C to 76°C significantly reduced the required inactivation time.

Several studies on virus inactivation by hydrogen peroxide have been reported. Lund (1963) obtained a 99% inactivation of poliovirus (Saukett strain) in about 6 hours with 0.3% (3,000 mg/l) hydrogen peroxide. Mentel and Schmidt (1973) worked with rhinovirus (types 1A, 1B, and 7).

TABLE 20

Contact Times Necessary for Hydrogen Peroxide (H_2O_2) to Effect 99% Inactivation of Various Concentrations of Escherichia coli, Salmonella typhi, and Staphylococcus aureus[a]

Test Microorganism	Initial Concentration, bacteria/ml	Contact Time Required for 99% Inactivation by Hydrogen Peroxide, min		
		30 mg/l	60 mg/l	90 mg/l
Escherichia coli	10^2	[b]	[b]	~360
	10^4	[b]	[b]	[b]
	10^6	[b]	[b]	~360
Salmonella typhi	10^2	~60	>30<45	<10
	10^4	>300	>45<60	30
	10^6	>300	>60	>45<60
Staphylococcus aureus	10^2	[c]	[b]	>15<30
	10^4	[c]	[b]	~60
	10^6	[c]	[b]	~180

---

[a] Data from Yoshpe-Purer and Eylan, 1968. Organism grown on nutrient agar (24-hour culture), suspended in saline, and exposed to varying concentrations of hydrogen peroxide in 1 liter of tap water, pH≈6.5, at room temperature (not specified).

[b] Inactivation in 300 min was less than 90%.

[c] No data reported.

They found that while a 1.5% (15,000 mg/l) concentration required approximately 24 minutes for 99% inactivation, equivalent inactivation occurred in 4 minutes with a concentration of 3% (30,000 mg/l).

Information is lacking on the effect of hydrogen peroxide on protozoa and helminths in water.

In none of the studies cited above is there any indication that the dosage of hydrogen peroxide was measured other than by the volumetric addition of hydrogen peroxide. No measurements of residual were reported, although Yoshpe-Purer and Eylan (1968) claimed that a residual was present for up to 13 days as indicated by inactivation of added doses of bacteria.

Mechanism of Action

No studies have specifically identified the mechanism of action of hydrogen peroxide. Spaulding et al. (1977) believed that the hydrogen peroxide molecule itself was not responsible for the action but, rather, that the free hydroxyl radical ($OH^{\bullet}$) that it produced was the specific inactivating agent. They claimed that the catalytic effect of iron or copper ions supported this theory. Yoshpe-Purer and Eylan (1968) also reported that free radicals were important but that sufficient catalyzing metal ions were available either from the tap water that they used or from the bacterial cells themselves.

Conclusions

Because of its relatively high cost and the high concentrations that are required to achieve disinfection in reasonable time, hydrogen peroxide is not a generally satisfactory disinfectant for drinking water.

Research Recommendations

If further research is to be conducted, the stability of hydrogen peroxide and its residual effect in the presence of organic material and other substances in water or distribution systems should be investigated. Parallel studies using chlorine and hydrogen peroxide, separately and in conjunction, should be conducted.

IONIZING RADIATION

Ionizing radiation may be electromagnetic or particulate. As used for disinfection or sterilization, electromagnetic radiation may be UV, gamma, or X-rays, and the particles may be alpha or beta or neutrons, mesons, positrons, or neutrinos. This discussion is limited to gamma rays and beta particles (or electrons).

Although there is extensive literature on the use of ionizing radiation in the preservation of food and other materials (Silverman and Sinskey, 1977), little information is available on water treatment.

Application

Laboratory studies of destruction of microorganisms are generally conducted by exposing small containers holding the test suspensions to the shielded source of ionizing radiation. However, in a practical application, the shielded source must be designed to permit relatively thin sheets of flowing liquid to be exposed. This is particularly important for high energy electrons which have a less penetrating power than gamma rays. The MIT study (1977) included a field demonstration in which approximately 380,000 l/day (plant (minimum dosage of 400,000 rads) was designed and operated. The energy source was a 50 kW electron accelerator operated at 850,000 V. Sludge was pumped under the electron beam through a drum system that produced a layer that was 1.2-m wide and 2-mm thick. The entire operation was conducted in a concrete vault to shield the operators against X-rays, which are produced incidentally. Shielding of workers is a major requirement both for gamma ray and high energy electron sources.

Analytical Methods and Residual

No residual is produced by ionizing radiation. Consequently, dosage has been measured exclusively. The MIT (1977) report and Silverman and Sinksey (1977) summarized analytical methods including calorimetry, photoluminescent dosimetry, colorimetry, and the use of ionization chamber instruments.

Biocidal Activity

Efficacy Against Bacteria

The first study of ionizing radiation in the treatment of water was reported by Dunn (1953). In addition to reviewing the literature and providing some discussion on ionizing radiation, he studied the use of a 1,000-Ci source of cobalt-60 (providing gamma rays) and a Van de Graaff generator (providing high energy electrons). Working with natural waters and wastewaters that were not chemically or bacteriologically characterized or controlled, he exposed samples to a constant radiation flux (2,000 R/minute from the cobalt-60 or electrons from 3-MV operation of the Van de Graaff generator). Varying only the time of exposure, Dunn found that it took 0.125 minutes (dosage 250,000 R) with the cobalt-60 source to reach 95.4% to 99.999% inactivation of total initial numbers of bacteria (as measured by a plate count at 37°C).

Ridenour and Armbruster (1956) studied the effect of a 10-kCi source of cobalt-60 (3,000 R/minute) on a variety of natural waters, wastewaters and pure cultures (approximately 2 x 10^7 organisms per milliliter) of 10 different organisms. They found that a dosage of 100,000 roentgen equivalent physical (rep) reduced the count of all species tested by ≥99%. (In order of increasing resistance, the species were: Enterobacter aerogenes, Escherichia coli, Shigella flexneri, Salmonella typhi, S. sonnei, Salmonella sp., Staphylococcus aureus, S. paratyphi B, Streptococcus faecalis, and Bacillus subtilis.) With river water, a dosage of from 50,000 to 100,000 rep reduced the total bacterial count and the coliform index by at least 99%, but 150,000 rep was required to reduce the streptococcus index by 99%. Varying the pH (5, 7, and 8.5) had no effect on the rate of inactivation.

Also using cobalt 60 (1,100 Ci), Lowe et al. (1956) exposed pure cultures of bacteria that were suspended in double distilled water and sterile settled sewage. The concentration of microorganisms was approximately 1x10^6/ml. Although they did not report exposure time, they summarized their exposure data in rads (radiation absorbed dose). (See Table 21.)

There have been more studies on the disinfection of wastewater than on disinfection of drinking water. Ballantine *et al.* (1969) and Compton *et al.* (1970) considered ionizing radiation not as good as other available methods but, with increased emphasis on water reclamation (Eliassen and Trump, 1973) and the use of high energy electrons (MIT, 1977; Wright and Trump, 1956), the prospects for practical application seem improved. In the MIT (1977) study, which is the most systematic one conducted to date, washed cells or spores were suspended in 0.067-M phosphate buffer (1 x 10^8 cells/ml) and exposed to high energy electrons from a Van de Graaff generator. The approximate dosages, in rads, required for a 99% inactivation of the organisms tested were: *Escherichia coli* (K12), 38,000; *Salmonella typhimurium* (LT2), 24,000; *Micrococcus* sp., 35,000; *Aspergillus niger* (spores), 78,000; *S. typhimurium* (24), 100,000; *Streptococcus fecalis*, 300,000; and *Clostridium perfunges* (spores), 400,000. The investigators found that the solids in the wastewater had no effect on disinfection.

Of the factors affecting disinfection, oxygen was most important. For example, for *E. coli* K12, the dosage required for 99% inactivation was more than doubled when an atmosphere of air was replaced by one of nitrogen, and

TABLE 21

Dosages of Cobalt-60 Irradiation Necessary for 99% Inactivation of Microorganisms in Distilled Water[a]

Test Microorganism	Dosage Required, rad
Bacillus subtilis var *niger*	3.5×10^5
Mycobacterium smegmatis	1.4×10^5
Escherichia coli	6.5×10^4
Micrococcus pyogenes var *aureus*	5.8×10^4
E. coli phage T3	3.2×10^4

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[a] Data from Lowe *et al.*, 1956.

almost halved when an air was replaced by an oxygen-rich atmosphere.

Efficacy Against Viruses

The inactivation of viruses does not appear to be influenced by the source of the ionizing radiation (Lea, 1955). It is affected by factors that are similar to those described above for bacterial inactivation (Ballentine *et al*., 1969). Lowe *et al*. (1956) found that 32,000 rads were required for 99% inactivation of *E. coli* phage T3 (see Table 21). In the MIT study (1977), from 300,000 to 420,000 rads were required for 99% inactivation of coxsackievirus B3, poliovirus 2, echovirus 7, reovirus 1, and adenovirus 5 (in order of increasing sensitivity), each suspended in 0.05 M glycine at pH 7.0.

Efficacy Against Parasites

The MIT study (1977) appears to be the only one in which the effect of ionizing radiation on both protozoa and helminths was investigated; however, no quantitative data were reported. Brannan *et al*. (1975) found that to attain a 90% reduction of embryonation of *Ascaris lumbricoides* eggs, a dosage of approximately 30,000 rads was required.

Mechanism of Action

In a review of the literature, Silverman and Sinskey (1977) summarized the mode of action of disinfection by ionizing radiation. Two effects are recognized: the direct effect in which the primary cellular target is DNA that is damaged by the energy released from the ionizing radiation, and the indirect effect, which is associated with the production of such substances in the cell menstruum as hydrogen peroxide (H_2O_2), organic peroxides, and free radicals. Oxygen is important because it reacts with electrons and radicals, which in turn react to form hydrogen peroxide or ozone (O_3).

Conclusions

Ionizing radiation can disinfect water effectively; however, the large source, shielding, and relatively thin exposure layers that are required create difficult engineering and safety problems. The complex technology may limit application to large facilities that can provide for adequate safeguards. The absence of residual disinfection is also restrictive.

Research Recommendations

Any further research should address practical methods of delivering the ionizing radiation to the water mass.

Basic engineering design, construction, and operation data should be developed.

POTASSIUM PERMANGANATE

Potassium permanganate ($KMnO_4$) is a strong oxidizing agent, which was first used as a municipal water treatment chemical by Sir Alexander Houston of the London Metropolitan Water Board in 1913. In the United States, it was used in Rochester, N.Y. in 1927 and in Buffalo, N.Y. in 1928. Since 1948 it has been used more widely in waterworks as an algicide (Fitzgerald, 1964; Kemp et al., 1966), as an oxidant to control tastes and odors (Spicher and Skrinde, 1963; Welch, 1963), to remove iron and manganese from solution (American Water Works Association, 1971; Shull, 1962), and, to a limited extent, as a disinfectant (Cleasby et al., 1964).

The relatively limited information concerning disinfection with potassium permanganate is subject to criticism because there have been no studies of the effects of organic constituents of the medium (test system) or destruction of a variety of organisms, especially pathogens.

Analytical Methods and Residual

Concentrations of potassium permanganate can be determined readily by iodometric titration using sodium thiosulfate ($Na_2S_2O_3$) as a titrant or by direct titration with ferrous sulfate. Manganese can be determined by atomic absorption spectrophotometry, or the permanganate ion

(MnO_4^-) can be measured colorimetrically (Standard Methods, 1976). The minimum detectable concentration of manganese by the colorimetric procedures, using a 100 ml sample, is 50 µg/l. A concentration of 0.05 mg/l or greater can be detected visually by the pink color that is imparted to the water (Welch, 1963).

Production and Application

Crystalline potassium permanganate is highly soluble in water (2.83 g/100 g at 0°C). In waterworks, it is prepared usually as a dilute solution (1% to 4%) and applied with a chemical metering pump (American Water Works Association, 1971). It also may be added as a solid using conventional dry-feed equipment.

Reduction of the permanganate ion produces insoluble manganese oxide (Mn_3O_4) hydrates. To prevent distribution of turbid water or of water that will cause unsightly staining of plumbing fixtures, potassium permanganate most often is applied as a pretreatment that is followed by filtration. For example, addition of potassium permanganate to a finished water to maintain a residual in a distribution system is unacceptable because of the pink color of the compound itself or the brown color of the oxides.

Biocidal Activity of Potassium Permanganate

Cleasby et al. (1964) and Kemp et al. (1966) summarized the few scientific references to disinfection by potassium permanganate that were published earlier than 1960. Although some bactericidal activity was indicated, no quantitative data were presented and the early reports disagreed as to its value.

The most systematic study of disinfection by potassium permanganate has been made by Cleasby et al. (1964). They worked exclusively with *Escherichia coli* (prepared as a lactose broth culture) and studied the effect of potassium permanganate at doses of 1 to 16 mg/l at pH values of 5.9, 7.4, and 9.2, temperatures of 0°C and 20°C, and contact times of 4 to 120 minutes. They concluded that bacterial inactivation was relatively ineffective, but slightly better at the higher temperature, and that increasing pH decreased disinfection rates. Table 22 summarizes in tabular form their data, which were presented originally in graphic fashion.

At least two patent applications involve the use of potassium permanganate as a disinfectant for water in swimming pools (Heuston, 1972; Seidel, 1973). Seidel's patent covered use of a concentration of 0.1 to 0.2 mg/l in a recirculating system including filtration through quartz gravel. She claimed removal of bacteria and algae, but the specific merit of potassium permanganate in her system cannot be determined from the patent application. Heuston

TABLE 22

Concentrations and Contact Times Necessary for Potassium Permanganate to Effect 99% Inactivation of a 48-Hour *Escherichia coli* Lactose Broth Culture[a]

Contact Time Required for 99% Inactivation by Potassium Permanganate, min							
1 mg/l	2 mg/l	4 mg/l	8 mg/l	12 mg/l	16 mg/l	pH	Temperature, °C
45	*c*	*c*	*c*	10	5	5.9	0
b	*c*	*c*	*c*	*b*	115	7.4	
c	*c*	*c*	*c*	*c*	*c*	9.2	
95	45	15	15	10	5	5.9	20
b	*c*	*b*	30	30	25	7.4	
c	*c*	*c*	*c*	*c*	*b*	9.2	

[a] Data from Cleasby *et al.*, 1964.

[b] Inactivation in 120 min was less than 90%.

[c] No data reported.

(1972) proposed a tablet containing 0.001 g of potassium permanganate per 0.2 g tablet to give a dose of 1 mg/l. Because the tablet includes potassium iodide (KI) and other oxidizing agents, it is impossible to assess the specific killing action of potassium permanganate.

A number of studies on inactivation by potassium permanganate have been reported. However, these dealt largely with the mode of action of potassium permanganate in viral inactivation (Lund, 1963, 1966) or at efforts to control human diseases (Peretts _et_ _al_., 1960; Schultz and Robinson, 1942; Wagner, 1951), animal (Derbyshire and Arkell, 1971), or plant diseases (Eskarous and Habib, 1972; Hughes and Steindl, 1955). They provide few clear-cut quantitative data on biocidal activity of the compound.

Information is lacking on the effect of potassium permanganate on protozoans and helminths in water.

Mechanism of Action

The mechanism of action of potassium permanganate has not been defintively identified. From the stuides that have been conducted (Lund, 1963, 1966) it may be presumed that it exerts its disinfection activities by oxidizing compounds that are involved in essential cellular functions. However, Lund (1963) questioned the suggestion that the oxidative process was the mechanism for poliovirus inactivation, but was unable to exclude it as a possibility.

Conclusions

The relatively high cost, ineffective bactericidal action, and the aesthetic unsuitability of maintaining a residual in the distribution system make potassium permanganate a generally unsatisfactory disinfectant for drinking water.

Research Recommendations

Additional studies on potassium permanganate are hardly warranted but in the unlikely event that further research on its biocidal activities is conducted, it would be desirable to study the effect of organic material on its disinfection and the effect of the compound on organisms other than *E. coli*. To relate disinfection efficiency to more conventional practices, parallel studies using chlorine should be conducted.

SILVER

Silver as a metal has been known for millennia, and its use as a water disinfectant dates back to the Persian king Cyrus. The term, oligodynamic, which describes the killing effect of small concentrations, was coined by von Naegeli in 1893, but it is unscientific and its use should be rejected. The antibacterial action of silver and silver nitrate ($AgNO_3$) was noted first by Raulin in 1869. Since the late 19th century, but especially since World War II, there have been considerable efforts to exploit the use of silver as a disinfectant, particularly for individual (home) water systems and swimming pools. Silver has been used both as a salt, most commonly silver nitrate, or as metallic silver, either bound in filter beds, generated by electrolytic devices, or applied as a colloidal suspension.

The relatively limited information concerning disinfection with silver has been seriously criticized because accurate measurement of low concentrations, i.e., <200 μg/l, has been difficult, a suitable neutralizing agent has not always been incorporated in test protocols, and adsorption of silver on surfaces of test vessels has confounded some studies. Despite these limitations, silver has been used in parts of Europe and Japan as a water disinfectant.

Production and Application

In water treatment, silver has been applied principally by dissolving the metal or by incorporating a silver compound in a filter medium, often an activated carbon filter. Romans (1954) has described older processes and patents. Davies (1976) reviewed processes for treating swimming-pool water in which silver was added as a soluble salt and the silver ions were kept oxidized by the addition of persulfate; metallic silver was deposited on an activated carbon filter; silver was released from solid silver electrodes, which were alternately made anodic and cathodic; silver was released from an activated carbon filter containing silver and supplemented by passing the water through anodic silver screens; and silver was released from a pair of silver-copper alloy electrodes by applying a low reversing voltage.

During the past 6 years, dozens of patents have been issued in Germany, Spain, South Africa, India, and the United States for devices or systems for adding silver to home drinking water systems or swimming pools. The home systems are most frequently combinations of activated carbon to remove tastes and odors and silver to prevent bacterial growth on the filter.

Because of the low solubility of silver, the dose is usually less than 50 μg/l. Presumably this is reduced rapidly because of the adsorption of silver to surfaces, but there is no information on the silver residual in treated water. However, the maximum contaminant level (MCL) is 0.05 mg/l (USEPA, 1975).

Analytical Methods

Until recently there have been no satisfactory techniques for measuring silver at the µg/l level. Using a dithizone colorimetric method, the minimum detectable quantity of silver is 200 µg/l (Standard Methods, 1976). Using an atomic absorption spectrophotometric method, the detection limit is 10 µg/l (USEPA, 1974), and if a heated graphite furnace is used, the detection limit is reduced to 0.005 µg/l (Rattonetti, 1974).

Biocidal Activity

One of the earliest studies was conducted by Just and Szniolis (1936) who found that 100 µg of silver per liter disinfected water within 3 to 4 hours, that silver nitrate and metallic silver were equally effective, and that histopathological changes occurred in rats that were given water containing 400-1,000 µg silver/l for 100 days. Other systematic studies of silver disinfection have been conducted by Renn and Chesney (1953-1956), Wuhrmann and Zobrist (1958), and Chambers and Proctor (1960). These studies have been summarized by Woodward (1963). Table 23, adapted from Wuhrmann and Zobrist (1958), shows that in the concentrations used, silver acted slowly, but that there were increases in the rate of bacterial inactivation with increasing temperature and pH. Chambers and Proctor (1960) obtained similar results, but the inactivation rates of Renn and Chesney (1953-1956), who used comparable concentrations of silver, were faster.

Both Wuhrmann and Zobrist (1958) and Chambers and Proctor (1960) found that the presence of phosphate (at 60 mg/l) slowed bacterial inactivation. Because phosphate is typically found at much lower concentrations in drinking water, this observation has little practical significance, but it may account for disparate results in some laboratory studies.

Increasing water hardness slowed bacterial inactivation. According to Wuhrmann and Zobrist, an increase of 3 minutes was required to achieve 99.9% bacterial inactivation (at 20°C and pH 7.0) for each 10 mg/l increase in hardness. Likewise, chlorides interfered with the action of silver. At 10 mg/l they increased the inactivation time by 25% and at 100 mg/l, by 70%.

The source of silver seemed to be irrelevant to the inactivation rate because silver either added as silver nitrate or dissolved from metallic silver gave approximately equal results.

In most studies of the disinfection of water with silver, Escherichia coli has been the test organism. Wuhrmann and Zobrist also tested a Salmonella species and found it to be at least as sensitive as E. coli. Yasinskii

TABLE 23

Concentrations and Contact Times Necessary for Silver to Effect 99% Inactivation of *Escherichia coli* in 0.005 M Phosphate Buffer[a]

Contact Time Required for 99.9% Inactivation by Silver as Silver Nitrate, min					
0.01 mg/l	0.03 mg/l	0.09 mg/l	0.27 mg/l	pH	Temperature, °C
1,010	837	156	53	6.3	5
466	214	81	34	7.5	
268	109	58	18	8.7	
831	344	144	32	6.3	15
316	177	63	21	7.5	
216	100	38	13	8.7	
1,210	152	68	20	6.3	25
423	86	32	13	7.5	
203	40	20	8	8.7	

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[a] Data from Wuhrmann and Zobrist, 1958

and Kuznetsova (1973) observed that 90 µg/l inactivated *Vibrio comma* (1 x 10^6 cells/ml) in 30 minutes or 22.5 µg/l in 60 minutes.

Fair (1948) and Harrison (1947) suggested that silver salts were ineffective against cysts of *Entamoeba histolytica* but the data provided in these reports were limited. By contrast, Newton and Jones (1949) observed that electrolytically produced silver in tap water gave greater than 99% cyst inactivation in 1 hour and highly variable residual concentrations that ranged between 17 and 33 mg/l at pH's of 9 to 9.8. When silver nitrate in distilled water at 0.5 mg/l was used, 4 to 6 hours were required for a 90% to 99% inactivation. At 5 mg/l, a >99% inactivation occurred in 3 hours, and at 30 mg/l, a >99% inactivation occurred in 1 to 2 hours.

Chang and Baxter (1955) used 150 mg/l of silver nitrate (95 mg/l Ag^+) with contact lines of 1 to 6 minutes. They concluded that cysticidal activity was only moderate compared to that of iodine. Apparently, there are no data on silver as a virucidal agent in water except for one study by Lund (1963), who decreased poliovirus activity by 2.5 logs in 4 hours with a concentration of approximately 68 mg/l.

A good general review of silver and its compounds and a more thorough medical examination of their applications was published by Grier (1977), who concluded that there would be a "significant place for silver compounds in the prevention and treatment of at least some bacterial diseases."

Mechanism of Action

Romans (1954) summarized the mechanism of so-called oligodynamic activity: "However, there is much difference of opinion as to the form of the active principle, as to the mechanism of its action, and the value of the results obtained. Some authorities believe that the active form is a positively charged ion, some think it is a complex ion, others a salt and still others think it acts by formation of proteinates or merely as a catalyst. These are only a few of the ideas that have been expressed with formidable experimental support."

Chang (1970) attributed a direct action to silver in the nonreversible formation of silver-sulfhydryl complexes that could not function as hydrogen carriers:

$$2R\text{-}SH \text{ ---> } \underset{\substack{\text{(normal}\\ \text{sulfhydryl)}}}{R\text{-}S\text{-}S\text{-}R} + H_2 \qquad (30)$$

$$R\text{-}SH + Ag^+ \text{ ---> } \underset{\substack{\text{(inactive}\\ \text{silver}\\ \text{complex)}}}{R\text{-}SAg} + H^+ \qquad (31)$$

He considered silver to be bacteriostatic as well as bactericidal. This would explain the relatively long contact times that are required for antibacterial activity at the concentrations that are normally used.

Zimmerman (1952) demonstrated that low concentrations of silver do not enter the cell but are adsorbed onto the bacterial surface just as silver tends to be adsorbed on other surfaces (Chambers and Proctor, 1960). According to Chang (1970) the adsorbed silver ions must immobilize the dehydrogenation process because bacterial respiration takes place at the cell surface membrane.

Conclusions

As a disinfectant, silver may be applicable to home treatment systems and swimming pools. These applications may be more effective in keeping filters free from bacteria than in actually inactivating organisms that are suspended in the water. This is because of the relatively low rate of solution of silver in water and its low biocidal activities.

Woodward (1963) ably summarized the situation: "The high cost of silver will limit it to speciality uses. The fact that silver does not impart taste, odor, or color to water makes it attractive for use. Its slow bactericidal action, although a disadvantage in some situations, may be an advantage in others, particularly where water is stored for long times before use, as on shipboard. Until some of the uncertainties regarding silver are resolved, it would be prudent to use it as a drinking water disinfectant only in situations where substantial factors of safety can be provided and where the bactericidal effectiveness of the procedure can be monitored."

Silver and its compounds are weak, costly disinfectants that are unsuitable for use in municipal drinking water supplies. To achieve acceptable disinfection in a reasonable time would require concentrations exceeding the MCL of 0.05 mg/l.

Research Recommendations

Now that adequate chemical analytical techniques are available for measuring low concentrations of silver, it would be desirable to conduct disinfection studies in which both dose and residual are measured accurately. To relate disinfection efficiency to more conventional practices, parallel studies using chlorine should be conducted.

ULTRAVIOLET LIGHT

Electromagnetic radiation, in wavelengths from 240 to 280 nm, is an effective agent for killing bacteria and other microorganisms in water (Luckiesh and Holladay, 1944). Conveniently, from a practical point of view, from 30% to more than 90% of the energy emitted by a low pressure mercury arc, which is enclosed in special UV transmitting glass, is emitted at a wavelngth of 253.7 nm (Anonymous, 1960; Childs, 1962; Luckiesh and Taylor, 1946).

Two basically different physical arrangements are commonly used for the application of UV light to water. In one, lamps are placed above the solution to be disinfected at the apex or focus of parabolic or elliptical reflectors. For this purpose, aluminum is preferred because of its high reflectance for the germicidal 253.7 nm wavelength (Luckiesh and Taylor, 1946). Although this is an efficient way of applying UV radiation to water, the open nature of the structure can permit contamination. Furthermore, it must operate at atmospheric pressure. Tubular reactors are more common in water treatment because they are sealed and operate under pressure.

To increase intensities and permit higher flow rates, multiple lamp reactors are being designed and put into use. These units generally contain lamps that are positioned parallel to the flow of water through them. A recent patent (Wood, 1974) describes an apparatus using a water film approximately 0.64 cm thick and a flow of liquid that is perpendicular to the lamp in a baffled system. This type of unit is designed for disinfection of fluids with low UV transmittance. For liquids with high transmittance like drinking water, such a design is inefficient because insufficient depth is available for absorption of UV and a large fraction of the radiant energy is dissipated as heat, when the UV is reflected on the walls of the contactor.

In units containing lamps that are surrounded by water, an insulation space must be provided to maintain their efficiency. The maximum efficiency of modern cold cathode lamps is near 40°C, dropping off to a 50% output at 24°C and 65°C. Lamps are normally placed in sleeves that are made of high silica glass or quartz in order to maximize transmission at the 253.7 nm wavelength. Solarization or opacity can develop in the sleeve as it deteriorates with age. The sleeves must be cleaned regularly for efficient functioning.

Another consideration in the mechanical design of UV contactors is the degree of agitation that is provided and the plug flow characteristics of the contactor. In all disinfection systems that require several orders of magnitude of disinfection, short circuiting or non-plug flow characteristics of contactors, which bypass a part of the flow, limit the efficiency of the process. It was recognized by Cortelyou et al. in 1954 that the degree of agitation in the UV disinfection of water is important in bringing the target microorganism into close proximity to the UV source where the intensity is highest.

Dose

The dose, D, of electromagnetic radiation that is applied to a solution is commonly measured as the intensity of radiant energy input, I_0, at the lamp surface or at some given distance from the lamp (I_0 being expressed as $\mu W/cm^2$), multiplied by the time of exposure, t, in seconds (Luckiesh and Holloday, 1944):

$$D = I_0 t \ \mu W.sec/cm^2 \tag{32}$$

Another measurement of dose is chemical actinometry (Calvert and Pitts, 1966). In this method a photochemical reaction with a known quantum yield is used to measure the intensity (quanta per second) of light that is absorbed by the actinometry solution. Knowing the time of exposure, the volume of solution, and the volume of sample analyzed, the moles of photochemical reaction per unit of time can be related through the quantum yield to the Einsteins or moles of photons per second that are absorbed by the fluid. From the wavelength of radiation, λ, and the area of lamp surface, the average intensity at the lamp surface can be determined.

$$E = hc/\lambda = 1.2 \times 10^7/\lambda \ W{\bullet}sec/Einstein \ nm \tag{33}$$

Neither of these measurements of energy input, I_0, per unit area of lamp exposed considers the change in intensity through the depth of exposed fluid and the solid angle over which this energy interacts. Recently, attempts have been made to correct the decrease in intensity with depth in studies of wastewater disinfection where this problem is acute (Roeber and Hoot, 1975; Severin, 1978; Venosa et al., 1978). These studies have shown that the usual water quality indices, such as chemical oxygen demand (COD), color, and turbidity, do not adequately predict the loss of intensity through the solution. The direct measurement of UV transmittance has been successful. In spite of these difficulties, Huff et al. (1965) suggested that color at a maximum level of 5 units, iron at 3.7 mg/l, and turbidity up to 5 units did not decrease treatment efficiency below acceptable limits in a unit with a maximum water depth of approximately 7.5 cm.

Biocidal Dose

The biocidal dose of UV energy consists of the intensity of UV energy that is adsorbed at the reactive site within the microbe over the time of interaction. The biocidal dose is then a function of the energy input from the UV source into the solution, dispersion of the energy as a function of distance from the source, the depth of the fluid between the organisms and the source as well as its adsorptivity, and, finally, the losses and reflection of UV light within the contactor. All of these factors determine the actual intensity of radiation that is available to the microorganism at any one point within the contactor (Childs, 1962; Luckiesh and Holladay, 1944; Luckiesh et al., 1944).

Quite early in the study of the batericidal action of UV light, Gates (1929) found that the relation between the intensity of incident energy and time required for bacterial destruction were not of equal importance in determining experimental disinfection. However, Hoather (1955) found that when one corrects for the transmitted intensity of radiation, taking into consideration the absorptivity of the water and dispersion as a function of distance from the source, the required time of exposure is inversely proportional to the calculated intensity of radiation penetrating the water for a given degree of inactivation. Finally, Oliver and Cosgrove (1975) used chemical actinometry and a pulsed laser to show that their coliform and streptococcal inactivation in wastewater depended only on the total dose that is delivered and not on the dose rate or intensity of light that impinged on the sample. Thus, the same number of $\mu W \cdot sec/cm^2$ provided the same disinfection regardless of the time and intensity that were used to produce that dosage.

Bacterial spores have also been studied with chemical actinometry. For certain stocks, the rate at which the energy was deposited affected the degree of response (Powers et al., 1974). Powers and his colleagues observed that the effect of the dose rate was not due to geometric factors or intensity variations with depth and solution absorptivity. This explains many earlier results. They did find that dependence on dose rate was seen only for some of their stocks of spores, it was not as dramatic with monochromatic-filtered light of a wavelength of 253.7 nm, and it was observed only at very low doses. The explanations usually given for these low-dose effects are photochemical back reactions, enzymatic repair, and photoreactivation mechanisms (Derring and Setlow, 1963; Harm, 1968).

Residual

UV radiation produces no residual. Therefore, monitoring and control of disinfection efficiency are more difficult for UV than for chemical disinfectants.

However, this is not a major problem because disinfection can be controlled by adjusting the contact time and the UV energy that is transmitted through the solution. The major disadvantage is the lack of a tracer for insuring the integrity of the distribution system.

Biocidal Activity

UV disinfection follows Chick's Law of kinetics (Luckiesh and Holladay, 1944):

$$-\log N/N_0 = \frac{I \times t}{Q} \qquad (34)$$

where N is the number surviving at time, t, of an original population, N_0, that has been exposed to an intensity, I. Q is the dose for 1 log survival, one lethal unit exposure, or a lethe.

The variables discussed above interfere with the accurate determination of the average intensity and time of exposure in a practical UV contactor. Consequently, there is considerable disagreement in the literature concerning the absolute magnitude of Q for the microorganisms that have been studied.

Of the Gram-negative bacteria, *Escherichia coli* is consistently more resistant to disinfection by UV than are the *Salmonella* and *Shigella* species that have been studied (Cortelyou *et al*., 1954; Katawaba and Harada, 1959). The vegetative forms of the Gram-positive bacteria were more resistant than *E. coli*. Kawabata and Harada found *Streptococcus faecalis* nearly three times more resistant than *E. coli*, while *Bacillus subtilis* was four times more resistant. The spores of *B. subtilis* were six times more resistant than *E. coli*.

Luckiesh and Holladay (1944) reported that a dosage of 2,400 $\mu W \cdot sec/cm^2$ at a wavelength of 254 nm produced one lethe of disinfection of *E. coli*. Huff *et al*. (1965) studied a two-lamp shipboard disinfection unit with an approximately 75-cm long, 20-cm diam unbaffled stainless steel cylinder. Their dosages were generally quite high, ranging from 3,000 to 11,000 $\mu W \cdot sec/cm^2$ at a maximum water depth of approximately 19 cm. At the lowest dosage, there was a 0.02% to 0.04% survival of E. coli which, assuming Chick's Law, gives a dosage of less than 400 μW•sec/cm²/lethe. They also found approximately 2 logs less spore inactivation with the same dose as required for vegetative cells. They reported more than 4 logs of inactivation of polio, echo-, and coxsackievirus with 4,000 μW•sec/cm². Based on these results, the U.S. Department of Health, Education, and Welfare issued a policy statement on April 1, 1966 stating criteria for the acceptability of UV disinfecting units as a minimum dosage of 16,000 μW•sec/cm² with a maximum water depth of approximately 7.5 cm.

Morris (1972) has compared the dose of 254 nm UV energy required to inactivate a range of microorganisms suspended in droplets of buffer solution on an aluminum surface. Their results are shown in Table 24 expressed as μW•sec/cm²/lethe.

Required dosages reported by Morris (1972) are similar in magnitude to those found by Huff et al. (1965). Both Huff's values and those of Morris (1972) are lower than those of Luckiesh and Holladay (1944) partly because of the additional dose of UV that was provided by reflections from the contactor and aluminum surface which produce an estimated dosage approximately twice the values given. No studies of the action of UV light on parasitic helminths and protozoa in the treatment of drinking water appear to have been reported.

TABLE 24

Ultraviolet Energy Necessary to Inactivate Various Organisms[a]

Test Microorganism	Lethal Dose ($\mu W.sec/cm^2$)
Escherichia coli	360
Staphylococcus aureus	210
Serratia marcescens	290
Sarcina lutea	1,250
Bacillus globigii spores	1,300
T3 coliphage	160
Poliovirus	780
Vaccinia virus	30
Semliki Forrest virus	470
EMC virus	650

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[a] Data from Morris, 1972.

Mechanism of Action

Inactivation by UV light is believed to act through the direct absorption of UV energy by the microorganism, causing a molecular rearrangement of one or more of the biochemical components that are essential to the organism's functioning (Loofbourow, 1948). The major site of UV absorption in microorganisms is the purine and pyrimidine components of the nucleoproteins. Since the relative efficiency of disinfection by UV energy as a function of wavelength follows the absorption spectrum of these chromophore groups and because the first law of photochemistry states that the light that is absorbed by a molecule can produce photochemical change, Calvert and Pitts (1948) concluded that the mechanism of action of UV occurs through the progressive biochemical change that is produced primarily in the nucleoproteins. Witkin (1976) has reviewed both UV mutagenesis and inducible DNA repair. The major specific mechanisms that have been suggested for UV damage involve the reversible formation of pyrimidine hydrates and pyrimidine dimers. Breaks in the bonding structure, known as nicking, also occur. The older literature has been reviewed by Reddish (1957).

The repair of damaged nucleoprotein with light of a wavelength longer than that of the damaging radiation is commonly referred to as photoreactivation (Witkin, 1976). This process, originally identified by Kelner (1949), has been studied extensively. It occurs in the visible wavelength range from 300 to 550 nm (Jagger, 1960; Kelner, 1949). In addition, repair of nucleoproteins that have been damaged by UV radiation can also occur in the dark. The mechanisms of repair of damage to DNA caused by UV radiation are generally thought to involve induced enzymes (Witkin, 1976), but repair has also been observed in inactive systems for which photochemical back reactions have been suggested (Powers *et al.*, 1974).

Conclusions

UV light produces no residual. Therefore, if a residual is desired another disinfectant must be used. Current technology is limited to the use of UV light in small systems. Requirements for equipment maintenance limits widespread adoption of UV light for drinking water disinfection.

SUMMARY

Tables 25 and 26 present significant characteristics of the methods that are considered for disinfection of drinking water.

The biocidal activity of various disinfectants can be compared conveniently through the numerical value of the product (c.t) of the concentration of disinfectant (c) multiplied by the time of exposure (t) that is required to achieve a particular degree of inactivation (e.g., 99%) under similar conditions of pH, temperature, etc. The lower its c.t product, the more effective the disinfecting action of a particular agent.

Table 27 displays c.t products for the methods that are regarded as the major possibilities for drinking water disinfection. The c.t products for similar inactivation under the same conditions obtained for other possible agents (ranked less promising by this or other criteria) are up to several orders of magnitude greater than those shown in Table 27, e.g., 33,000 and 10^6 for inactivation of *E. coli* and poliovirus 1 by hydrogen perioxide, respectively. Therefore, they were not included.

TABLE 25

Summary of Major Possible Disinfection Methods for Drinking Water

Disinfection Agent[a]	Technological Status	Efficacy in Demand-free Systems[b]			Persistence of Residual in Distribution System
		Bacteria	Viruses	Protozoan cysts	
Chlorine[c]	Widespread use in U.S. drinking water				Good
As hypochlorous acid (HOCl)		++++	++++	++	
As hypochlorite ion (OCl^-)		+++	++	NDR[d]	
Ozone[c]	Widespread use in drinking water outside U.S., particularly in France, Switzerland, and the province of Quebec	++++	++++	++++	No residual possible
Chlorine dioxide[c,e]	Widespread use for disinfection (both primary and for distribution system residual) in Europe; limited use in U.S. to counteract taste and odor problems and to disinfect drinking water	++++	++++	NDR[d]	Fair to good (but possible health effects)
Iodine	No reports of large-scale use in drinking water				Good (but possible health effects)
As diatomic iodine (I_2)		++++	+++	+++	
As hypoiodous acid (HOI)		++++	++++	+	
Bromine	Limited use for disinfection of drinking water	++++[f]	++++[f]	+++[f]	Fair
Chloramines	Limited present use on a large in U.S. drinking water	++	+	+	Excellent

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[a] The sequence in which these agents are listed does not constitute a ranking.

[b] Ratings: ++++, excellent biocidal activity; +++, good biocidal activity; ++, moderate biocidal activity; +, low biocidal activity; ±, of little or questionable value.

[c] By-product production and disinfectant demand are reduced by removal of organics from raw water prior to disinfection.

[d] Either no data reported or only available data were not free from confounding factors, thus rendering them not amenable to comparison with other data.

[e] MCL 1.0 mg/l because of health effects (Symons et al., 1977).

TABLE 26

Summary of Minor Possible Disinfection Methods for Drinking Water

Disinfection Agent[a]	Technological Status	Efficacy in Demand-Free Systems[b]			Persistence of Residual in Distribution System
		Bacteria	Viruses	Protozoan cysts	
Ferrate	No reports of use in drinking water	++	+++	NDR[c]	Poor
High pH conditions (pH 12-12.5)	No reports of large-scale use in drinking water	+++	+++	NDR[c]	Feasibility restricted as consumption of high pH water not recommended
Hydrogen peroxide	No reports of large-scale use in drinking water	±	±	NDR[c]	Poor
Ionizing radiation	No reports of use in drinking water	++	++	NDR[c]	No residual possible
Potassium permanganate	Limited use for disinfection	±	NDR[c]	NDR[c]	Good, but aesthetically undesirable
Silver[d]	No reports of large-scale use in drinking water	+	NDR[c]	+	Good, but possible health effects
UV light	Use limited to small systems	+++	+++	NDR	No residual possible

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[a] The sequence in which these agents are listed does not constitute a ranking.

[b] Ratings: ++++, excellent biocidal activity; +++, good biocidal activity; ++, moderate biocidal activity; +, low biocidal activity; ±, of little or questionable value.

[c] Either no data reported or only available data were not free from confounding factors, thus rendering them not amenable for comparison with other data.

[d] MCL 0.05 mg/l because of health effects (Symons et al., 1977).

TABLE 27

Comparative Efficacy of Disinfectants in the Production of 99% Inactivation of Microorganisms in Demand-Free Systems[a]

Disinfection Agent	E. coli pH	E. coli Temperature, °C	E. coli c.t[b]	Poliovirus 1 pH	Poliovirus 1 Temperature, °C	Poliovirus 1 c.t[b]	Entamoeba histolytica cysts pH	Entamoeba histolytica cysts Temperature, °C	Entamoeba histolytica cysts c.t[b]
Hypochlorous acid	6.0	5	0.04	6.0	0	1.0	7	30	20
				6.0	5	2.0			
				7.0	0	1.0			
Hypochlorite ion	10.0	5	0.92	10.5	5	10.5		NDR[c]	
Ozone	6.0	11	0.031	7.0	20	0.005	7.5-8.0	19	1.5[d]
	7.0	12	0.002	7.0	25	0.42			
Chlorine dioxide	6.5	20	0.18	7.0	15	1.32			
	6.5	15	0.38	7.0	25	1.90		NDR[c]	
	7.0	25	0.28						
Iodine	6.5	20-25	0.38	7.0	26	30	7.0	30	80
	7.5	20-25	0.40						
Bromine		NDR[c]		7.0	20	0.06	7.0	30	18
Chloramines									
Monochloramine	9.0	15	64	9.0	15	900		NDR[c]	
	9.0	25	40	9.0	25	320			
Dichloramine	4.5	15	5.5	4.5	15	5,000		NDR[c]	

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[a] Conditions closest to pH 7.0 and 20°C were selected from studies discussed in the text.

[b] Values for other conditions and agents appear in the text along with discussions of the cited studies.
Concentration of disinfectant (mg/l) times contact time (min).

[c] Either no data reported or only available data were not free from confounding factors, thus rendering them not amenable for comparison with other data.

[d] This value was derived primarily from experiments that were conducted in tap water; however, some parallel studies in distilled showed essentially no differences in inactivation rates.

CONCLUSIONS

Table 28 provides the current status of theoretically possible methods for disinfecting drinking water. To derive the conclusions in the table the specific biocidal activity data summarized in Table 27 were considered as well as information (or lack of it) on the practical application and reliability of the methods.

Chlorination, ozonization, and the use of chlorine dioxide come closest to meeting the criteria desired. At this time none of the other possibilities considered can substitute for techniques presently used to disinfect drinking water.

The ultimate choice between methods will require weighing the characteristics detailed in this evaluation against the nature of by-products to be expected from the use of the particular method and the potential toxicities of the by-products. Evaluations of these aspects of drinking water disinfection, also carried out by the Safe Drinking Water Committee, are reported in parallel to this study, and should be consulted before final concusions are drawn.

TABLE 28

Status of Possible Methods for Drinking Water Disinfection

Disinfection Agent	Suitable Inactivating agent	Limitations	Suitability for Drinking Water Disinfection[a]
Chlorine	Yes	Efficacy decreases with increasing pH; affected by ammonia or organic nitrogen	Yes
Ozone	Yes	On-site generation required; no residual; other disinfectant needed for residual	Yes
Chlorine dioxide	Yes	On-site generation required; interim MCL 1.0 mg/l	Yes
Iodine	Yes	Biocidal activity sensitive to pH	No
Bromine	Yes	Lack of technological experience; activity may be pH sensitive	No
Chloramines	No	Mediocre bactericide, poor virucide	No[b]
Ferrate	Yes	Moderate bactericide; good virucide; residual unstable; lack of technological experience	No
High pH conditions	No	Poor biocide	No
Hydrogen peroxide	No	Poor biocide	No
Ionizing radiation	Yes	Lack of technological experience	No
Potassium permanganate	No	Poor biocide	No
Silver	No	Poor biocide; MCL 0.05 mg/l	No
UV light	Yes	Adequate biocide; no residual; use limited by equipment maintenance considerations	No

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[a] This evaluation relates solely to the suitability for controlling infectious disease transmission. See conclusions.

[b] Chloramines may have use as a secondary disinfectant in the distribution system in view of their persistence.

RESEARCH RECOMMENDATIONS

The lack of data, particularly data on pathogens or data that are amenable to comparison, on the biocidal efficacy of various disinfectants needs to be remedied. Particular emphasis should be placed on the methodology for determining biocidal activity and defining those parameters that need to be controlled for the study to yield data for comparative purposes. Disinfection efficacy studies should be conducted with actual pathogens (bacteria, viruses, and cysts of *Giardia lamblia* and *Entamoeba histolytica* as well as with model systems. Careful control of the character and concentration of the disinfecting species and of the degree of aggregation and other characteristics of the test organisms (or cysts) are required in these tests.

Studies should be conducted to define the mechanisms resulting in the different responses to disinfectants of laboratory-acclimated cultures and organisms that have been freshly isolated from natural conditions or that are resistant to specific disinfectants for other reasons. Techniques should be developed to facilitate accurate predictions from laboratory data to disinfectant suceptibilities under operating conditions in treatment plants.

There are no practical assays for many human viruses of known or suspected importance, e.g., hepatitis A virus and rotaviruses. Assay models should be developed for such viruses, and studies should be conducted on their suceptibilities to disinfection. Support is also needed for research that would result in better appraisals of the significance to human health of the presence of viruses in water supplies. Such studies should include investigation of the causative agents that are involved in the large number of illnesses presently characterized as "gasteroenteritis of unknown etiology" and of the doses of various enteroviruses that are required to establish infections.

In investigations of factors affecting biocidal activity in full-scale treatment plant operations priority should be given to studying more thoroughly the effects of turbidity (both organically and inorganically caused) on disinfection.

It should be determined if any data are available from treatment plants on the effectiveness of ozone or chlorine dioxide in disease prevention, where these methods of disinfection are not used sequentially or, in the case of ozone, with subsequent chlorination. If such data, free of complications, are available, they should be compared with the records on the efficacy of chlorination disinfection.

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